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# Microbial ecology of waterborne pathogens in Sus scofra and Odocoileus virginianus in the Jackson Bienville Wildlife Management Area

Jaymes Hunter Collins

# MICROBIAL ECOLOGY OF WATERBORNE PATHOGENS IN *SUS SCOFRA* AND *ODOCOILEUS VIRGINIANUS* IN THE JACKSON-BIENVILLE WILDLIFE MANAGEMENT AREA

by

Jaymes Hunter Collins, B.S.

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We hereby recommend that the dissertation prepared under our supervision by

**Jaymes Hunter Collins, B.S.**

entitled **Microbial Ecology of Waterborne Pathogens in *Sus scofra* and *Odocoileus virginianus* in the Jackson-Bienville Wildlife Management Area**

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Supervisor of Dissertation Research

Head of Department
**School of Biological Sciences**
Department

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**Approved:**

Director of Graduate Studies

Dean of the College

**Approved:**

Dean of the Graduate School

# ABSTRACT

Previous studies have demonstrated that feral swine (*Sus scofra)* are significant reservoirs for a number of pathogens that present a potential threat to wildlife and humans. Despite this, few studies have gone beyond quantifying the incidence of these pathogens to further probe their ecology within a specific habitat or ecosystem.

Overall, the objective of this study was to characterize three potential reservoirs in a feral swine infested habitat; two ungulates, and one aquatic reservoir. Our study area was the Jackson-Bienville Wildlife Management Area (J-B WMA). We chose four waterborne bacteria: *Brucella* spp., *Leptospira interrogans*, *Salmonella enterica*, and *Helicobacter pylori*, and two waterborne protozoal pathogens: *Giardia lamblia* and *Cryptosporidium parvum* to assess in the J-B WMA. We developed a straightforward protocol to assess feral hog wallows which we recommend to others as a supplemental benchmark if they study feral swine.

Using PCR, we analyzed whole blood and fecal samples collected from feral swine (N=47) and white-tailed deer (N=49) within the J-B WMA for the following bacterial pathogens: *Brucella* spp., *Leptospira interrogans*, and *Salmonella enterica*, as well as two protozoans: *Giardia lamblia* and *Cryptosporidium parvum*. Sera from feral swine (N=47) and white-tailed deer (N=49) were also collected and tested for *Brucella* spp. and *Leptospira interrogans* using the Rose Bengal Test and Microscopic Agglutination Test (MAT) respectively. Feral swine stomach samples (N=16) were

collected and tested by PCR for the presence of a fourth bacterial pathogen, *Helicobacter pylori,* but no positives were documented. Water samples from feral swine wallows (N=20) were also collected and tested for the same pathogens using PCR.

Our results showed a high rate of incidence for each pathogen (except *H. pylori*) in feral hogs; and all pathogens were found to be present in many wallows as well. White-tailed deer tested positive for each bacterial pathogen, albeit at a lower rate, and none tested positive for either protozoal pathogen. Analysis of feral swine wallows showed they possess physical characteristics compatible with a sustained bacterial and protozoal presence. We have shown that feral swine in the J-B WMA are reservoirs for three bacterial and two protozoal pathogens included in this study.

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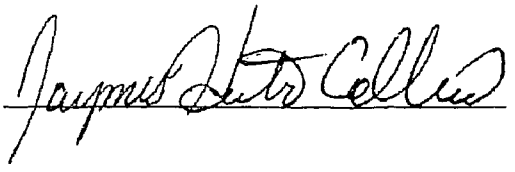
Author 

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# DEDICATION

This dissertation is dedicated to every person who has sacrificed their time and energy to help me during my time at Louisiana Tech University. The constant support from faculty and administrators, as well as friends and family, has made this possible. Words cannot express how grateful I am to each and every one of you.

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I came to Louisiana Tech and is one of the main reasons I decided to come here for graduate school. I would never have found my passion for teaching if he had not offered me a teaching assistantship when I started here, and for that I will always be grateful. He was always a reliable person to go to if I ever had problems or needed advice, which proved to be invaluable during difficult times.

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# CHAPTER 1

# INTRODUCTION

## 1.1 Background on Feral Hogs

Feral swine (*Sus scofra*) are an invasive species in the United States that have been designated a "high-risk species" due to how easily they establish themselves in an environment and the ecological and economic damage they cause thereafter (1). Feral swine possess many characteristics that contribute to their success as an invasive species. They are able to reproduce as early as six months of age and can produce up to two litters a year. Each litter can range from two to more than ten piglets at a time (2, 3). They are also resistant to a wide range of diseases, making them excellent reservoirs for these disease causing pathogens (4). They are opportunistic generalists in regards to their diet and habitat, which removes those barriers from limiting how large their home range can be (5). Feral swine are also highly intelligent and are able to quickly adapt to adverse conditions and environments. Perhaps the most influential variable that has led to feral swine becoming such a consequential invasive species is the human variable. Humans have played a major role in mobilizing feral swine ever further from whatever their current boundaries happen to be (6). Hunting pressure as well as live transport of feral swine have led to an alarming spread of feral swine in the United States within the last 20 years (Figure 1) (7).

**Figure 1:** Distribution of Feral Swine in the United States in 1988 (Left) and 2009 (Right) (7).

As their populations spread, feral swine leave a path of destruction in their wake. In the southern United States alone, feral swine are the leading cause of stream pollution, timber destruction, and wildlife competition (8, 9). A large group of feral swine can root and destroy as much as five acres of land in one night (6). It is estimated that the cost of damage caused by feral swine each year is close to 1.5 billion dollars in the United States alone (1). These factors necessitate increased efforts in controlling their expanding populations.

Another impetus for increased efforts in population control stems from the risks feral swine pose for transmitting diseases to native wildlife, livestock, and humans. Estimates are approximately 45 human and animal diseases are present in feral swine populations (4). A recent outbreak of *E. coli* OH:157 that killed three people traced back to feral swine feces on a spinach farm in California (10). This event, while tragic, serves to illustrate the consequences associated with unmanaged feral swine populations. While disease transmission directly from feral swine to humans is rare, knowledge of the

pathogens they harbor can be invaluable in risk assessment and management of feral swine populations.

Unfortunately, further insight into their potential for being pathogen reservoirs that cause human disease has not been heavily pursued. Studies have focused mainly on diseases that affect wildlife and domestic animals specifically. Feral swine could also be reservoirs for pathogens that have not previously been associated with their species. Many pathogens, such as *Helicobacter pylori* and Norovirus, have not had a definitive reservoir identified outside of humans (11, 12). Follow-up research on the significance of feral swine as hosts for human diseases is needed to educate the public on the health risks associated with feral swine.

A key component is still missing, however, for adequately assessing the risks feral swine pose for spreading disease. To date, there has been little to no research conducted on the mechanisms that allow feral swine to spread pathogens in their environment. Direct contact between feral swine and other wildlife, or with humans, is rare, which begs the question of how feral swine can spread disease. One possibility is through contamination of water sources, which could indirectly lead to feral swine infecting surrounding wildlife, domestic livestock, and humans that come into contact with contaminated water from feral swine.

## 1.2 Feral Hog Wallows

Feral swine present a number of risks to water quality in an environment, due in large part to the high number of pathogens they are known to carry. *Salmonella enterica, E. coli, Leptospira interrogans*, and *Brucella* spp. are a few examples of pathogenic bacteria feral swine potentially deposit into the watershed via excrement or urination into

swine wallows (13-15). Wallows are shallow bodies of water that feral swine use to regulate their body temperature; they do not have sweat glands. They also use these wallows to aid in removing ectoparasites from their bodies (16). These wallows often communicate with creeks or bayous and easily drain into the watershed during rain.

Because of this, wallows have the potential to be point sources for water contamination, as well as reservoirs for waterborne pathogens. Microbial sampling of streams and rivers would require concentration of large water samples whereas smaller samples taken directly from wallow water are more than satisfactory. However, studies on feral swine wallows as reservoirs for waterborne pathogens are almost non-existent. There are also few studies that have looked at the impacts feral hogs have on watersheds (13-15). This lack of information demonstrates a need for studies that focus on the effects feral swine have on water quality in their environment and the ways in which they are affecting water sources. Figure 1-2 shows an example of a feral swine wallow.

**Figure 1-2**: Feral swine wallow found in the Jackson-Bienville Wildlife Management Area.

## 1.3 Background on White-tailed Deer

White-tailed deer are an important and popular game mammal for hunters throughout the United States. They are also potential reservoirs for a number of diseases of humans as well as livestock and other wildlife (17). Because of their popularity with hunters as a game mammal, humans are at an increased risk for exposure, especially during field or other processing of deer. Knowledge of the types of diseases that are carried by white-tailed deer is critical in educating the public on pathogen-specific risks and appropriate handling precautions.

Another important aspect regarding diseases carried by white-tailed deer is specifics on the chain of infection, particularly significant origins of infections. Very little research has been done to trace the origin of diseases in white-tailed deer. This is odd considering how quickly a disease can spread within a population of white-tailed deer due to their highly social behavior within a group (18). Understanding how a population is initially infected can lead to improved efforts in controlling the spread of disease within white-tailed deer populations as well as to humans and other wildlife.

## 1.4 Background on Bacterial Pathogens in this Study

The increased spread of feral swine presents many issues; one of which is their potential to harbor diseases such as brucellosis, leptospirosis, and many others (4). Interest in studying feral swine as reservoirs for clinically relevant pathogens has been steadily increasing in recent years (4, 19-21). One pathogenic genus of bacteria that is being studied in feral swine is *Brucella*. *Brucella* spp. are gram-negative coccobacilli that act as facultative intracellular parasites. They can survive in extreme conditions and spread through ingestion, direct contact, and inhalation. A minimum infection dose,

either blood-borne or waterborne, can be as low as 10-100 organisms (21-28). These attributes have led to *Brucella* spp. being used as a potential weapon in biological warfare (29). This genus of bacteria also causes the disease brucellosis. In humans, this disease can cause severe joint and muscle pain and can last anywhere from a few weeks to a chronic lifetime condition. In swine and deer, this disease can have detrimental effects on reproductive capabilities, leading to potential population declines in both species.

Many studies have highlighted the risk feral swine pose for spreading *Brucella* spp. to surrounding wildlife as well as humans. One of the key factors in determining the risk for feral swine spreading *Brucella* spp. is the population density of feral swine in a given location (30). The prevalence of *Brucella* spp. can range from single digit percentages in a population with low density to as high as 60% for a population with high density (28, 30). Feral swine spread *Brucella* spp. many different ways, the most common being through urine and other bodily fluids excreted into their environment. For humans, the most common route of infection is through direct contact with an infected animal.

Serology is the most common method of *Brucella* spp. detection. There are many different serological techniques used to identify *Brucella* spp. including ELISA, fluorescence polarization assay, and the Rose Bengal test. The Rose Bengal test has been found to be the most cost-effective and specific test by a number of different groups (31). Previous studies have shown feral swine populations serologically positive for *Brucella* spp., with percentages ranging from 18% to over 50% (32, 33).

Direct detection of *Brucella* spp. with PCR is another technique that has seen an increase in usage (34-37). Although PCR is not frequently used for the clinical detection

of *Brucella* spp., it is considered more precise and sensitive than the Rose Bengal test. Previous researchers have determined that the Brucella specific transposon IS711 is the best target for PCR (34, 37). IS711 is found in all described species of *Brucella,* and can be used to identify specific species and biovars of *Brucella* based on characteristic insertion sites within their genome (37, 38).

*Leptospira interrogans* is another bacterial (spirochete) pathogen of interest in our studies because it fits into waterborne (and blood-borne) route of infection. In mammals, it is the cause of the disease leptospirosis. It thrives in tropical and sub-tropical regions and has a high potential for zoonotic spread (39, 40). One of the primary economic concerns with leptospirosis in livestock is the damage it can cause to the reproductive system. Leptospirosis leads to spontaneous abortions and declining reproductive rates in animals such as deer, cattle, and horses (41, 42).

Detection of *Leptospira interrogans* is commonly accomplished using either the Microscopic Agglutination Test (MAT) or traditional PCR. MAT is widely considered the gold standard for laboratory diagnosis of *Leptospira interrogans,* and it differentiates specific strains of *Leptospira interrogans* as well (43). However, using MAT to diagnose *Leptospirosis interrogans* is labor intensive and can be costly and time-consuming. PCR provides a faster, more efficient, way to diagnose *Leptospira interrogans*. The lipL32 gene has been shown to be the most effective target when using PCR to detect the presence of *Leptospira interrogans* (44, 45). This gene codes for the outer-membrane protein LipL32 that acts as a virulence factor during infection of *Leptospirosa interrogans* (46).

## 1.5 Background on Waterborne Intestinal Pathogens in this Study

*Helicobacter pylori* and *Salmonella* spp. are potentially waterborne bacterial pathogens carried by feral swine. *Helicobacter pylori* is of special interest because no definitive reservoir outside of humans has been identified. Recent research, however, provides strong evidence that feral swine may be an important reservoir of *Helicobacter pylori* (47-51). *Helicobacter pylori* is known to cause stomach ulcers and has been linked to an increased risk of stomach cancer (52, 53).

*Salmonella* spp. is another pathogen carried by feral swine that causes human disease in contaminated water, surfaces, or food. This pathogen, in particular, is a concern because of its high prevalence in feral swine populations and the ease in which it spreads within their populations (54, 55). PCR is currently the most common technique used in a laboratory setting to diagnose the presence of both *Salmonella* spp. and *Helicobacter pylori* (50, 54).

*Cryptosporidium parvum* and *Giardia lamblia* are common protozoal pathogens carried by feral swine (56, 57). Both of these protozoa have been labeled as "neglected diseases", causing a surge in research in their diagnosis, prevention, and treatment (58). Finding more effective ways to eliminate these pathogens from the environment has also been a major focus. *Cryptosporidium parvum*, in particular, is difficult to eliminate in the environment. It is resistant to extremely high levels of chlorination, levels well beyond those used to treat municipal water supplies, and can survive concentrations as high as 1000 mg/L of chlorine (59).

*Cryptosporidium parvum* causes the disease cryptosporidiosis, which is associated with severe diarrhea and can lead to death if not properly treated. A person can become infected with *Cryptosporidium parvum* through drinking contaminated water or through ingestion of contaminated food. A minimum infectious dose is estimated to be approximately 130-150 oocysts, which is the infectious form of *Cryptosporidium parvum* (60). *Cryptosporidium parvum* is difficult to detect due to the small size of the oocysts, making diagnosis difficult in places without advanced methods of detection. Selective staining and microscopy can be used for detection but more sensitive and precise methods, such as PCR and ELISA, have become the preferred method of detection.

Giardiasis is caused by *Giardia lamblia*, and is the most common parasitic infection worldwide. This disease can cause severe diarrhea and poor nutrient absorption (61). *Giardia lamblia* is spread primarily through the fecal-oral route in the infectious cyst form. Ingestion of contaminated water is usually the primary route of infection, although it can also be spread through contaminated food or direct contact. *Giardia lamblia* can persist in the environment for months at a time in its cyst form and has been shown to be infectious after surviving months in near freezing water (62).

Detection of *Giardia lamblia* is relatively simple compared to *Cryptosporidium parvum*. Immunological detection using ELISA is the most definitive method of detection, having a success rate of over 90% (63). However, this test is costly and is usually reserved for *Giardia lamblia* detection in a research setting. Microscopy is still used to detect *Giardia lamblia*, although it is mostly used as a screening method for more precise methods of detection. PCR remains the preferred method for detection due to its versatility and specificity (64-68).

# CHAPTER 2

# MATERIALS AND METHODS

## 2.1 Study Site

Our study site was the Jackson-Bienville Wildlife Management Area (J-B WMA). The J-B WMA is located approximately 12 miles south of Ruston, LA. It has two major access points off U.S. Highway 167 and Louisiana Highway 147. It includes approximately 25,000 acres of land managed by the Louisiana Department of Wildlife and Fisheries. It contains a number of different timber types, but is predominately covered by pine in areas other than bottomland. The bottomland areas contain a greater diversity of timber types including cypress, beech, and other hardwood timber. It contains a number of different habitat types due to timber diversity and the different areas that are managed specifically for certain species, such as quail and turkey. There is also a considerable amount of land dedicated to managing the red-cockaded woodpecker found in the J-B WMA. The red-cockaded woodpecker, one of the most ecologically important species found in the J-B WMA, is a federally endangered species.

The J-B WMA is open to the public and can be hunted during every major hunting season. White-tailed deer, squirrels, rabbits, and turkey are the primary game animals hunted. A major concern on the J-B WMA is the destruction of wild turkey nests by feral hogs. They also destroy plots of land that are reserved for timber restoration by

uprooting and killing tree saplings. Efforts to control the number of feral swine on the J-B WMA have increased in recent years but have so far been ineffective. A one-month long (February) season during which the use of dogs is allowed to hunt and trap feral swine has been in effect for several years in order to increase hunting pressure on feral swine populations. While some argue that this has been effective at controlling feral swine numbers, it could be argued that this has an overall negative impact on the spread of feral swine since this drives them further out into other areas. A map of J-B WMA is shown in Figure 2-1. Maps with the locations of captured feral swine and feral swine wallows that were included in this study were generated using the program ARCGIS.

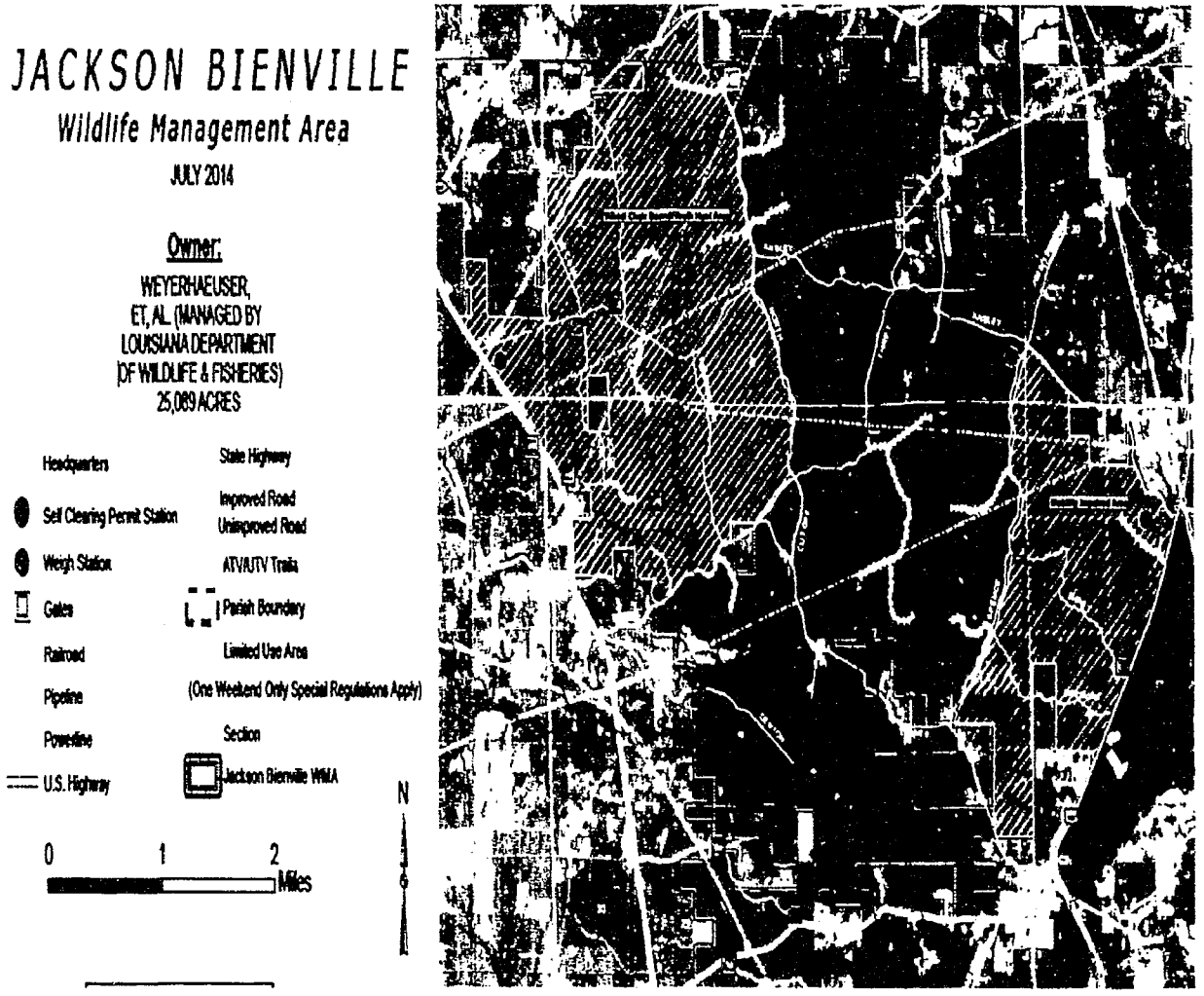


**Figure 2-1**: A map of the Jackson-Bienville Wildlife Management Area.

## 2.2 Feral Swine and White-tailed Deer Sample Collection

Feral swine were trapped using a "funnel gate" trap. A schematic of the trap used in this study is shown Figure 2-2. The traps were baited with corn and set using a trigger system that ensured the trigger only tripped when they are inside the trap. Captured swine were sacrificed to allow for sample collection. Samples collected from whitetail deer were acquired with assistance from hunters on the J-B WMA and local wildlife biologists. Whole blood, serum, and fecal samples were taken from 47 feral swine and 49 whitetail deer. Whole blood was collected into 4 ml vacutainer tubes containing an anti-coagulating agent. One ml aliquots were taken from these tubes and stored in 1.5 ml tubes at -20°C until needed. Serum was collected using 4 ml vacutainer tubes and spinning the whole blood at 5,000 RPM for 15 minutes. One ml aliquots of serum were collected from the spun blood and stored in 1.5 ml tubes at -20°C until needed.

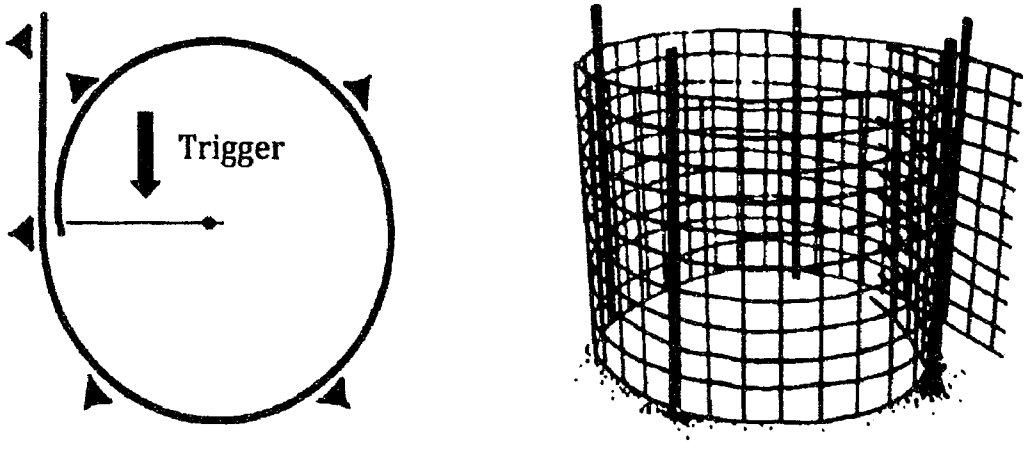


**Figure 2-2:** Schematic of traps used to capture feral swine in this study.

Fecal samples were fixed using Parasep® fecal parasite concentrators. These tubes contained a formalin-free fixative that allows for easier downstream applications. Whole stomachs were collected when possible and stored at -20° C until needed.

### 2.2.1 Determination of Characteristics of Samples

The age, weight, and gender of feral swine and white-tailed deer were collected from all samples used for this study. The age of feral swine was estimated by weight, which is a technique that has been used in previous studies (69). The age of white-tailed deer was determined by dental examination (70). SPSS statistical software was used to determine frequency of male and female gender as well as the age structure and weight within each group. Graphs of these results were also generated with SPSS.

### 2.2.2 DNA Extraction from Whole Blood Samples

DNA was extracted from whole blood samples using the IBI Scientific (Peosta, IA) DNA/RNA Extraction Kit by following the manufacturer's protocol. Briefly, a 200 µl aliquot of whole blood was digested with Proteinase K to break down cells and release genomic DNA. The DNA was then purified on the kit's mini column which has a silica-based membrane. Purified DNA was then eluted with 100 µl of the elution buffer provided in the kit, then quantified by $OD_{260}$. DNA samples were labeled and stored at -20°C until needed.

### 2.2.3 DNA Extraction from Fecal Samples

DNA was also extracted from approximately 25 mg fecal samples using IBI's DNA/RNA Extraction Kit and following the manufacturer's protocol. Quantification and storage were performed in the same manner as for whole blood DNA.

### 2.2.4 DNA Extraction from Feral Swine Stomach Samples

DNA was extracted from stomach samples using the same kit and protocol used for fecal samples. The sample itself was obtained by scraping the stomach mucosa with a sterile cotton swab until approximately 25 mg of viscous material was obtained.

Scrapings were done within several hours of death and samples were processed immediately. Samples were never refrigerated or kept on ice during transport because *H. pylori* is very temperature sensitive and will not survive refrigeration.

## 2.3 Feral Swine Wallow Sample Collection

Wallow water samples were collected in 50 ml conical tubes. Dissolved oxygen, temperature, pH, and water depth were measured on site for each wallow sampled. Sediment samples from each wallow were also collected to be analyzed using XRF to determine the elemental composition of each sample.

### 2.3.1 Coliform Enumeration of Feral Swine Wallow Samples

Wallow samples were filtered through a fine mesh sieve three times to remove any sediment or large debris. A 75-micron filter was used for a final filtration to capture any residual debris or fine sediment. A serial dilution of the filtrate (1:10, 1:100, 1:1,000) was performed in 100 µl of lactose broth. Each dilution was plated onto MacConkey agar plates and incubated at 37°C for 48-72 hours. This was done in triplicate for each sample. The following formula was used to determine the number of coliforms/100 ml:

Coliforms/100 ml = (Number of colonies counted) x 100 / Dilution Factor

### 2.3.2 DNA Extraction from Feral Swine Wallow Samples

A 1 ml aliquot of filtered wallow water was subjected to three freeze-thaw cycles at -80°C for 10 minutes and 60°C for 10 minutes respectively. The sample was then centrifuged at 14,000 x g for 5 minutes and the flow-through was discarded. DNA was then extracted using the same protocol for whole blood DNA extraction in Section 2.2.2. Purified DNA was tested for the presence of *Brucella* spp., *Leptospira interrogans*,

*Salmonella enterica*, *Cryptosporidium parvum*, *Giardia lamblia*, as well as human, ruminant and pig specific *Bacteroides*.

### 2.4 Bacterial Microbial Detection in Whole Blood Samples

PCR was used to analyze feral swine and whitetail deer genomic DNA from whole blood samples for the presence of *Leptospira interrogans* and *Brucella* spp. A combination of custom primers and previously published, validated primers were used for this part of the study and are listed in Table 1. The custom primer set for the detection of *Leptospira interrogans* targeted the gene LipL32. LipL32 is an outer membrane protein found in *Leptospira interrogans* and acts as a virulence factor in human infection. It also is highly conserved within the *Leptospira* genome, which makes it an excellent target for detection by PCR (33). The published *Leptospira interrogans* primer set is specific for Group B serovars of *Leptospira interrogans* and includes the serovars hardjo and canicola, which were included as targets in our serological assay (71). The custom primer set for *Brucella* spp. detection used in this study targeted the gene IS711. IS711 is an insertion sequence found exclusively in the *Brucella* genome. The variable number of occurrences and the position in which it occurs is species specific, which makes IS711 an excellent target for *Brucella* spp. detection and species specific identification using PCR (31). The published primer set for *Brucella* spp. targets the BS1330_II0657 locus of the *Brucella* genome (72). Each PCR reaction contained 6.5 µl of molecular grade water, 200 □g to 1 µg of genomic DNA extracted from feral swine whole blood in 5 µl of elution buffer, 100 □M of the desired primer set in 1 µl of molecular grade water, and 12.5 µl of 2x AMRESCO Hot Start Mix for a total volume of 25 µl.

Each PCR assay also included a positive and negative control reaction. The positive control contained 9.5 µl of molecular grade water, 200 ng to 1 µg of either *Leptospira interrogans* or *Brucella suis* DNA in 1 µl of molecular grade water, 100 □M of the desired primer set in 1 µl of molecular grade water, and 12.5 µl of 2x AMRESCO Hot Start Mix. The negative control contained 11.5 µl of molecular grade water, 100 □M of the desired primer set in 1 µl of molecular grade water, and 12.5 µl of 2x AMRESCO Hot Start Mix.

PCR conditions consisted of an initial denaturation at 94°C for 5 minutes to inactivate the enzyme bound to TAQ polymerase and allow for PCR to occur. After initial denaturation, an additional denaturation step occurred at 94°C for 30 seconds. The annealing step occurred at 61°C for 30 seconds, followed by an elongation step at 72°C for 1 minute. A final extension was performed at 72°C for 5 minute. These steps were repeated for 35 cycles for *Leptospira interrogans* assays and 40 cycles for *Brucella* spp. assays. Gel electrophoresis was used to visualize the PCR products. A 1.5% agarose gel was used for all gel electrophoresis assays.

**Table 1:** PCR Primers for Pathogen Detection

| Organism | Primer (5'-3') | Reference |
|---|---|---|
| *Brucella* spp. | F: CGGTGTATGGGAAAGGCT* | This |
| | R: CGTGGACTTTCGATATGGTG | Study |
| | F: TGGCTCGGTTGCCAATATCAATG* | (72) |
| | R: CGCGCTTGCCTTTCAGGTCTG | |
| *Leptospira interrogans* | F: AGGGACAAACGAAACCGTAA* | This |
| | R: ATTACGGCAGGAATCCAAAC | Study |
| | F: CGATGGAACCGATCCAATTA* | (71) |
| | R: CGTGACCTTTGTCAGTTACTCT | |
| *Salmonella enterica* | F: GCTGCGCGCGAACGGCGAAG* | (73) |
| | R: TCCCGGCAGAGTTCCCATT | |
| *Giardia lamblia* | F: CATCCGCGAGGAGGTCAA* | (66) |
| | R: GCAGCCATGGTGTCGATCT | |
| *Cryptosporidium parvum* | F:CAAATTGATACCGTTTGTCCTTCTG* | (66) |
| | R: GGCATGTCGATTCTAATTCAGCT | |
| *Helicobacter pylori* | F: CTAGCCCTGAACCCATTTA* | (50) |
| | R: CTAGCTGAAAGCCCTACCTTAC | |
| Pig Specific *Bacteroides* | F:CATGAATTTAGCTTGCTAAATTTGT | (20) |
| | R: ACCTCATACGGTATTAATCCGC | |
| Human Specific | F: TGTAAAACGACGGCCAGT* | (20) |
| *Bacteroides* | R: TACCCCGCCTACTATCTAATG | |
| Ruminant Specific | F: TGTAAAACGACGGCCAGT* | (20) |
| *Bacteroides* | R: CATCCCCATCCGTTACCG | |

*All forward primers had the M13 sequence (TGTAAAACGACGGCCAGT) added to the 5' end to simplify sequencing. The identity of all positive PCR amplicons (all primers) were confirmed by DNA sequencing (Eurofins Genomics, Louisville, KY).

## 2.5 Bacterial Microbial Detection in Serum Samples

Serum analysis for detection of *Leptospira interrogans* exposure was performed at the Veterinary Diagnostic Laboratory in Little Rock, Arkansas using the Microscopic Agglutination Test (MAT). The MAT assay tested for the presence of seven serovars of *Leptospira interrogans*. A titer between 1:100 and 1:400 (these are serum dilution titers, the amount of antigen remains constant) indicated past exposure to *Leptospira interrogans* only, while a titer >1:800 indicated an active infection. All results were verified by Jin Xie D.V.M, PhD.

Serological testing for exposure to *Brucella* spp. was conducted using the Rose Bengal test, also known as the *Brucella* CARD test. The CARD test is a rapid micro-agglutination assay to detect anti-*Brucella* antibodies present in serum. Thirty µl aliquots of *Brucella* spp. antigen and 30 µl aliquots of feral swine serum were thoroughly mixed together on a blank card using a sterile toothpick. A 30 µl aliquot of *Brucella* spp. positive bovine serum was used as a positive control. Once the antigen and serum sample were mixed, the card was rocked back and forth for five minutes to allow for sufficient reactivity. A positive reaction was indicated by agglutination of the antigen, forming a ring around the border of the mixture. An example of a positive reaction is shown below in Figure 2-3.

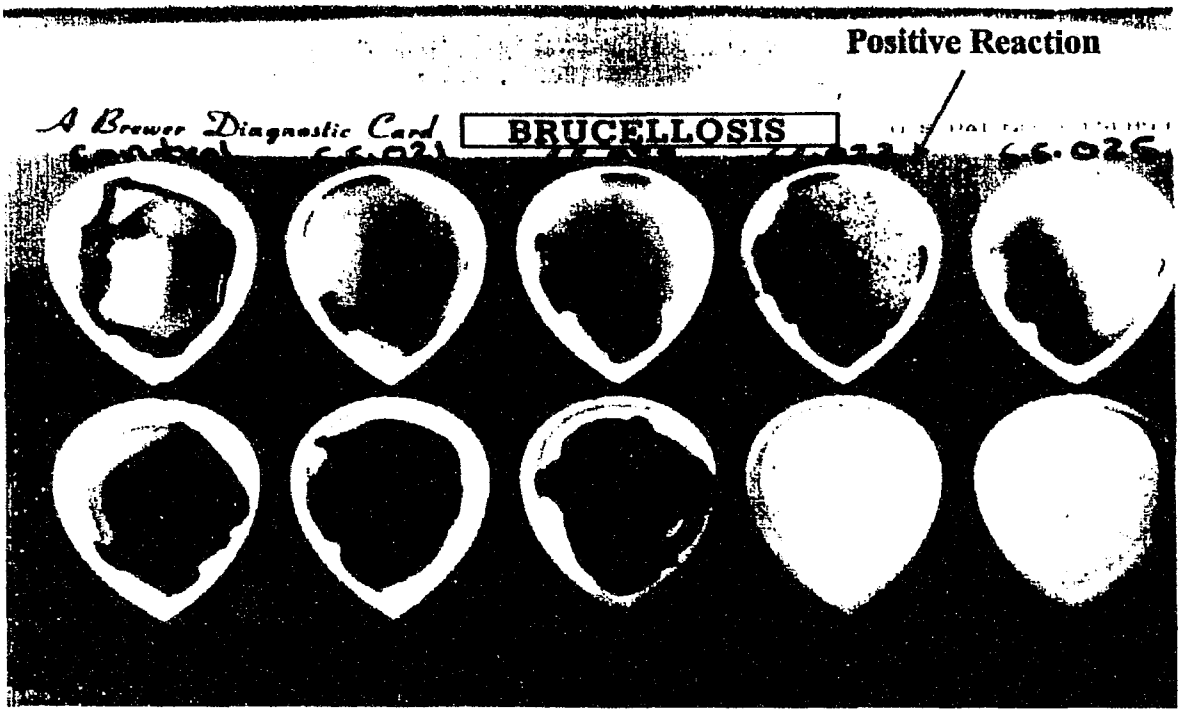


**Figure 2-3**: Example of the Rose Bengal test showing a positive reaction.

## 2.6 Bacterial Microbial Detection in Fecal Samples

PCR was used to analyze fecal samples for the presence of *Salmonella enterica*, *Cryptosporidium parvum*, and *Giardia lamblia*. Primers used to detect the presence of these pathogens are listed in Table 1. The *Salmonella enterica* primer set targets the gene invA, which is a virulence factor (73). The target of the *Cryptosporidium parvum* primer set is cowP, which is a cell wall protein and is regarded as a virulence factor (66). The *Giardia lamblia* primer set targets the gEno gene, which is specific to *Giardia lamblia* (66). Each PCR reaction consisted of 8.5 µl of molecular grade water, 3 µl of purified fecal DNA extracted from feral swine fecal matter, 100 □M of the desired primer set in 1 µl of molecular grade water, and 12.5 µl of 2x AMRESCO Hot Start Mix for a total volume of 25 µl.

Each PCR assay also included a positive and negative control reaction. The positive control contained 9.5 µl of molecular grade water, 1 ul of either *Salmonella enterica*, *Cryptosporidium parvum*, or *Giardia lamblia* DNA, 100 □M of the desired primer set in 1 µl of molecular grade water, and 12.5 µl of 2x AMRESCO Hot Starter Mix. The negative control contained 11.5 µl of molecular grade water, 1 µl of the appropriate primer set, and 12.5 µl of 2x AMRESCO Hot Start Mix.

PCR conditions for all pathogens being tested for consisted of an initial denaturation at 94°C for 5 minutes to inactivate the enzyme bound to TAQ polymerase and allow for PCR to occur. After initial denaturation, an additional denaturation step occurred at 94°C for 30 seconds. The annealing step occurred at 61°C for 30 seconds, followed by an elongation step at 72°C for 1 minute. A final extension was performed at 72°C for 5 minute. These steps were repeated for 35 cycles for all pathogens being tested.

Gel electrophoresis was used to visualize the PCR products. A 1.5% agarose gel was used for all gel electrophoresis assays.

### 2.6.1 *Cryptosporidium* Specific Staining of Oocysts and Microscopy

Staining and microscopy was used to test for the presence of *Cryptosporidium parvum* in all collected feral swine fecal samples. A protocol specific for staining *Cryptosporidium parvum* oocysts was modified from a published protocol (74). A thin smear of the fecal material was made on a standard microscope slide and heat fixed by placing the slide on a slide warmer set at 60°C for 10 minutes or until dry. The slide was then placed in a Coplin jar containing acidic alcohol (3% [vol/vol] HCl in methanol) and allowed to stand for 5 minutes. The slide was then removed from the acidic alcohol solution and the excess solution was rinsed off using de-ionized water. The slide was then placed in a Coplin jar containing safranin (1% [wt/vol]) in acidified water (pH 6.5) and microwaved at 650 watts for 1 minute. The slide was then removed and any excess stain was rinsed off using de-ionized water. The slide was then placed in a Coplin jar containing an aqueous solution of 1% [wt/vol] malachite green and allowed to stand for 1 minute. The slide was then removed and any excess stain was rinsed off using de-ionized water. A coverslip was mounted and the slide was observed using a Nikon microscope using the 100x objective.

## 2.7 Detection of *Helicobacter pylori* in Feral Swine Stomachs

PCR was used to analyze each stomach tissue sample for the presence of *Helicobacter pylori*. The primer set used to detect the presence of *Helicobacter pylori* is listed in Table 1. This primer set targets the gene cagA found in *Helicobacter pylori*.

CagA is a virulence factor for *Helicobacter pylori* infections and is exclusive to the *Helicobacter* genus (71). Each PCR reaction contained 6.5 µl of molecular grade water, 200 ng to 1 ug of genomic DNA extracted from feral swine whole blood in 5 µl, 100 □M final concentration of the *Helicobacter pylori* primer set in 1 µl molecular grade water, and 12.5 µl of 2x AMRESCO Hot Start Mix for a total volume of 25 ul. Only a negative control reaction was included in this assay. The negative control contained 11.5 µl of molecular grade water, 100 □□ final concentration of the *Helicobacter pylori* primer set in 1 µl of molecular grade water, and 12.5 µl of 2x AMRESCO Hot Start Mix.

The protocol for *Helicobacter pylori* detection consisted of an initial denaturation step at 94°C for 5 minutes. This was followed by 35 cycles of 94°C for 30 seconds, 61°C for 30 seconds, 72°C for 1 minute. A final extension was performed at 72°C for 5 minutes. All PCR products were visualized on a 1.5% agarose gel using gel electrophoresis.

### 2.8 Bacterial and Protozoal Microbial Detection in Feral Swine Wallow Samples

PCR was used to analyze DNA extracted from feral swine wallows for the presence of *Leptospira interrogans*, *Brucella* spp., *Salmonella enterica*, *Cryptosporidium parvum*, and *Giardia lamblia*. We also tested each wallow sample for human, ruminant, and pig specific *Bacteroides* to determine the source of microbial contamination. Each PCR reaction consisted of 6.5 µl of molecular grade water, 200 □g to 1 µg of purified DNA from feral swine wallow filtrate in 5 µl of elution buffer, 100 □M of the desired primer set in 1 µl of molecular grade water, and 12.5 µl of 2x AMRESCO Hot Start Mix for a total volume of 25 µl.

Each PCR assay also included a positive and negative control reaction. The positive control contained 9.5 µl of molecular grade water, 200 □g to 1 µg of either *Brucella* spp., *Leptospira interrogans*, *Salmonella enterica*, *Cryptosporidium parvum*, *Giardia lamblia*, human, ruminant, or pig specific *Bacteroides* DNA in 1 µl of molecular grade water, 100 nM of the desired primer set in 1 µl of molecular grade water, and 12.5 µl of 2x AMRESCO Hot Starter Mix. The negative control contained 11.5 µl of molecular grade water, 100 □M of the desired primer set in 1 µl of molecular grade water, and 12.5 µl of 2x AMRESCO Hot Start Mix.

PCR conditions for all pathogens being tested consisted of an initial denaturation at 94°C for 5 minutes to inactivate the enzyme bound to TAQ polymerase and allow for PCR to occur. After initial denaturation, an additional denaturation step occurred at 94°C for 30 seconds. The annealing step occurred at 61°C for 30 seconds, followed by an elongation step at 72°C for 1 minute. These steps were repeated for 35 cycles for all pathogens being tested except for *Brucella* spp. which was subjected to 40 cycles of the above steps. A final extension was performed at 72°C for 5 minutes. Gel electrophoresis was used to visualize the PCR products. A 1.5% agarose gel was used for all gel electrophoresis assays.

### 2.9 XRF Analysis of Feral Swine Wallows

Approximately 25 grams of sediment from each feral swine wallow were analyzed using X-Ray fluorescence. The sample was exposed to short-wave X-rays, which caused ionization of atoms in the sample to occur. Ionization occurs when an electron is ejected from an atom, potentially causing an atom in a higher orbital to fall into the now empty space left by the ejected electron. When this happens, energy is

released in the form of a photon. A detector in the XRF apparatus is able to measure the radiation emitted by the photon. The measure of radiation is element specific, which allows precise measurement of the quantity and identity of elements within a given sample. We used this technique to measure the level of heavy metals within our wallow samples, such as iron, copper, sulfur, and various other heavy metals that can impact microbial growth. Copper has been shown to negatively affect microbial growth (75). Sufficient iron is required for microbial growth and can affect the sustainability for microbial growth (76). We compared sediment samples taken from feral swine wallows to sediment taken near the wallows that had not been disturbed by feral swine to establish a baseline for comparison.

## 2.10 Correlations between Age, Weight, or Gender and Pathogen Prevalence

We determined a binomial logistic regression was the appropriate test to determine if there were any correlations between the age, weight, or gender of our feral swine and whitetail deer samples and pathogen prevalence. This test was performed using the SPSS statistical software. This technique was chosen due to the binary nature of our data (pathogen detected, yes or no). A p-value was generated for each parameter tested.

## 2.11 Correlations between Coliform Count and Pathogen Prevalence in Wallows

We also used binomial logistic regression to determine if there was a statistically significant correlation between the amount of coliform bacteria found in the wallow samples and the prevalence of the pathogens for which we were testing.

# CHAPTER 3

# RESULTS

## 3.1 Whole Blood Analysis

Our results showed that 11% (5 of 47) of feral swine samples tested positive for *Brucella* spp. Samples that tested positive for both *Brucella* spp. primer sets and by the Rose Bengal test were counted as positive. This was due to the difficulty in accurately detecting the presence of *Brucella* spp. using PCR. 10% (5 of 49) of white-tailed deer samples tested positive for *Brucella* spp. The same criteria used for designating a sample positive for *Brucella* spp. in feral swine was used for white-tailed deer.

Fifty-five percent (26 of 47) of feral swine samples tested positive for *Leptospira interrogans* using the custom primer set designed for this study. The published primer set for *Leptospira interrogans* detection showed 45% (21 of 47) of feral swine samples were positive for *Leptospira interrogans*. 6% (3 of 49) of white-tailed deer samples tested positive for *Leptospira interrogans* using both the custom primer set designed for this study and the published primer set. Figures 3-1 and 3-2 show agarose gels with PCR positive samples for *Brucella* spp. and *Leptospira interrogans* respectively. A graph showing a comparison of feral swine and white-tailed deer results is shown in Figure 3-3.

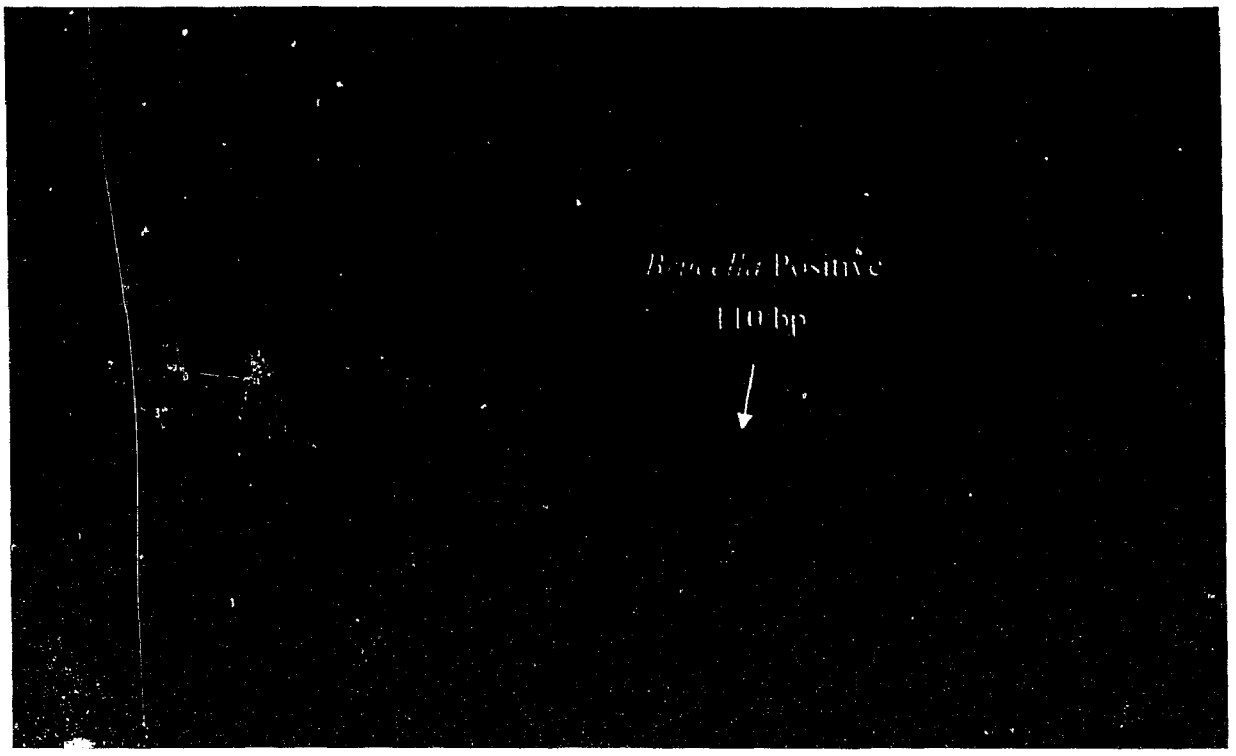


**Figure 3-1**: Agarose gel depicting a sample that was PCR positive for *Brucella* spp.


**Figure 3-2**: Agarose gel depicting a *Leptospira interrogans* positive sample.

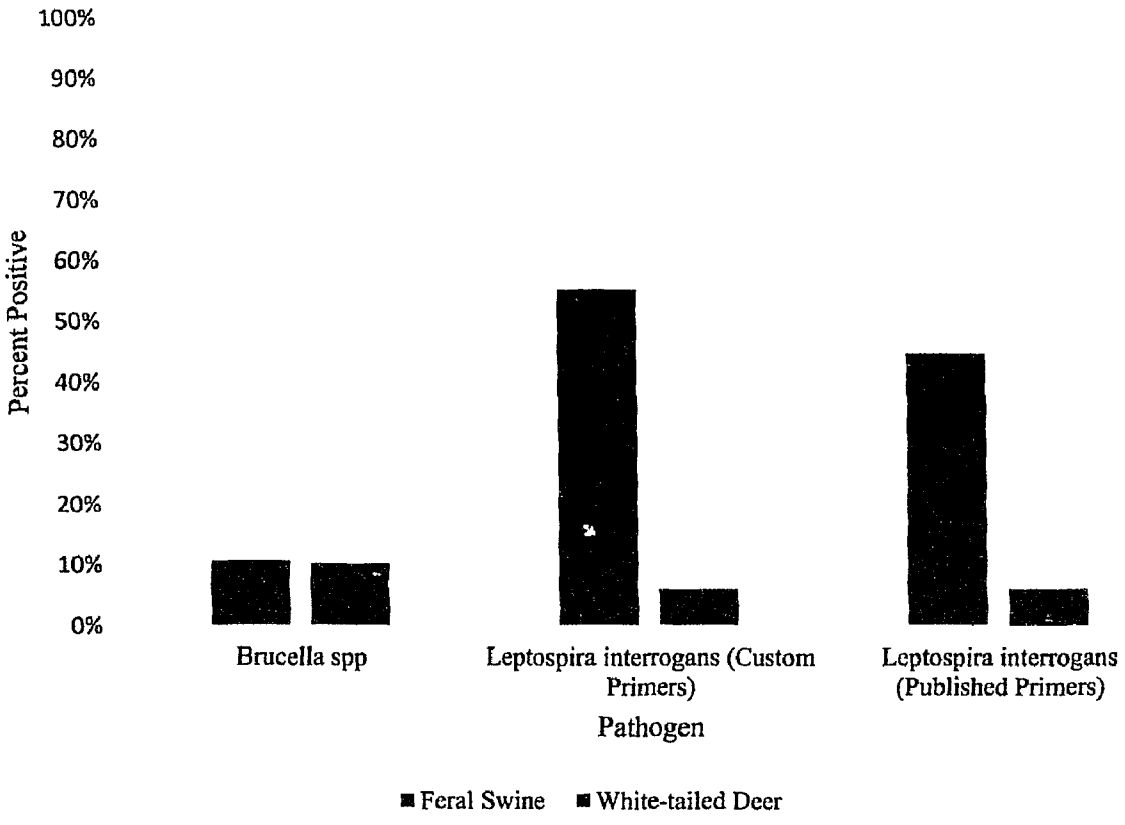


**Figure 3-3**: Percentage of feral swine and white-tailed deer whole blood samples that tested positive for *Brucella* spp and *Leptospira interrogans*.

## 3.2 Serum Analysis

Results from the MAT assay showed 57% (27 of 47) of feral swine samples tested positive for *Leptospira interrogans* exposure. This includes all serovars tested and all active *Leptospira interrogans* infections. Only 13% (6 of 47) tested positive for an active infection. 6.1% (3 of 47) of white-tailed deer serum samples tested positive for *Leptospira interrogans* exposure. MAT testing revealed that all positives in both hog and deer serum were due to exposure to the *hardjo* serovar of *Leptospira interrogans*. None of the white-tailed deer serum samples tested positive for an active infection. Graphs showing a comparison of feral swine and white-tailed deer results are shown in Figures 3-4 and 3-5.

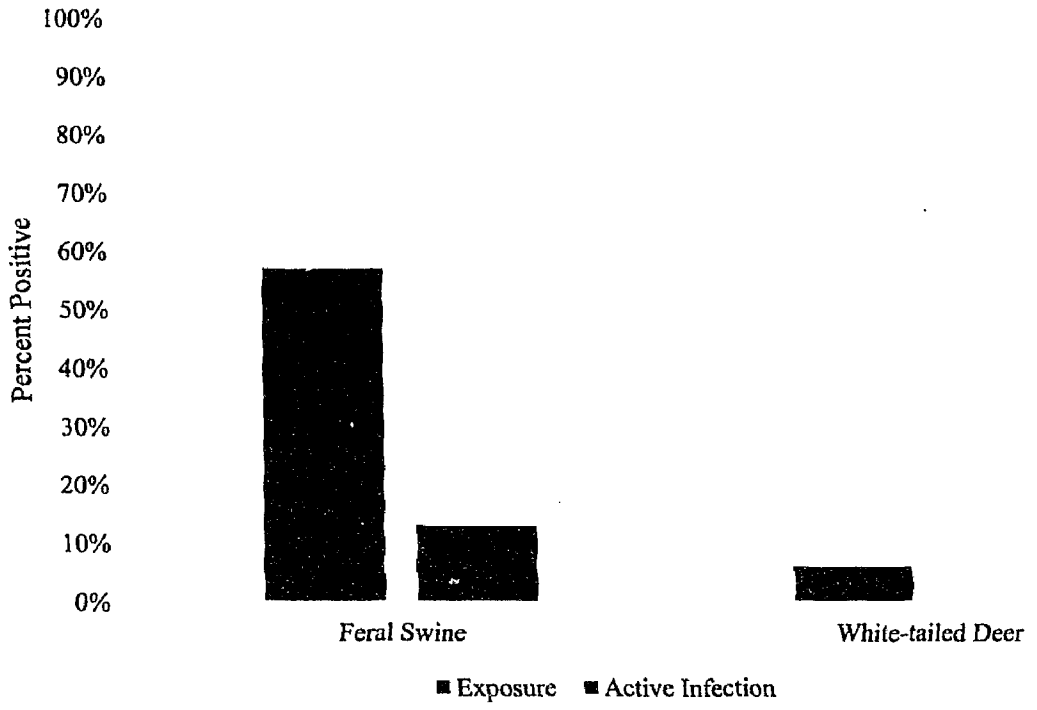


**Figure 3-4**: Percentage of feral swine and white-tailed deer serum samples that tested positive for *Leptospira interrogans* exposure and active infection.

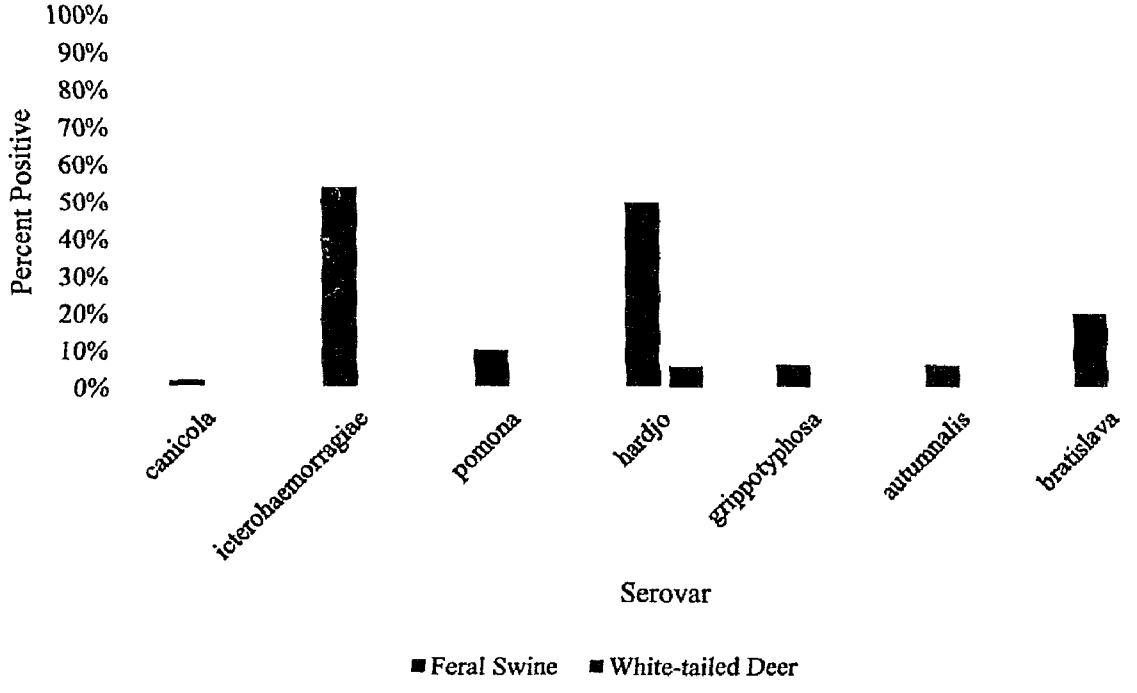


**Figure 3-5**: Percentage of feral swine and white-tailed deer samples that tested positive for each serovar of *Leptospira interrogans*.

### 3.3 Feral Swine Stomach Analysis

None of the stomach samples tested positive for *Helicobacter pylori*.

### 3.4 Fecal Analysis

Thrity-eight percent (18 of 47) of feral swine fecal samples tested positive for *Salmonella enterica*. DNA from *Giardia lamblia* and *Cryptosporidium parvum* was detected in 4.3% (2 of 47) and 8.5% (4 of 47) of samples respectively. Eight percent (4 of 49) of whitetail deer fecal samples tested positive for *Salmonella enterica*. DNA from *Giardia lamblia* and *Cryptosporidium parvum* was not detected in any of the whitetail deer fecal samples. Figure 3-6 shows an agarose gel with PCR positive samples for *Salmonella enterica, Giardia lamblia* and *Cryptosporidium parvum*. A graph showing a comparison of feral swine and white-tailed deer results from fecal analysis is shown in Figure 3-7.

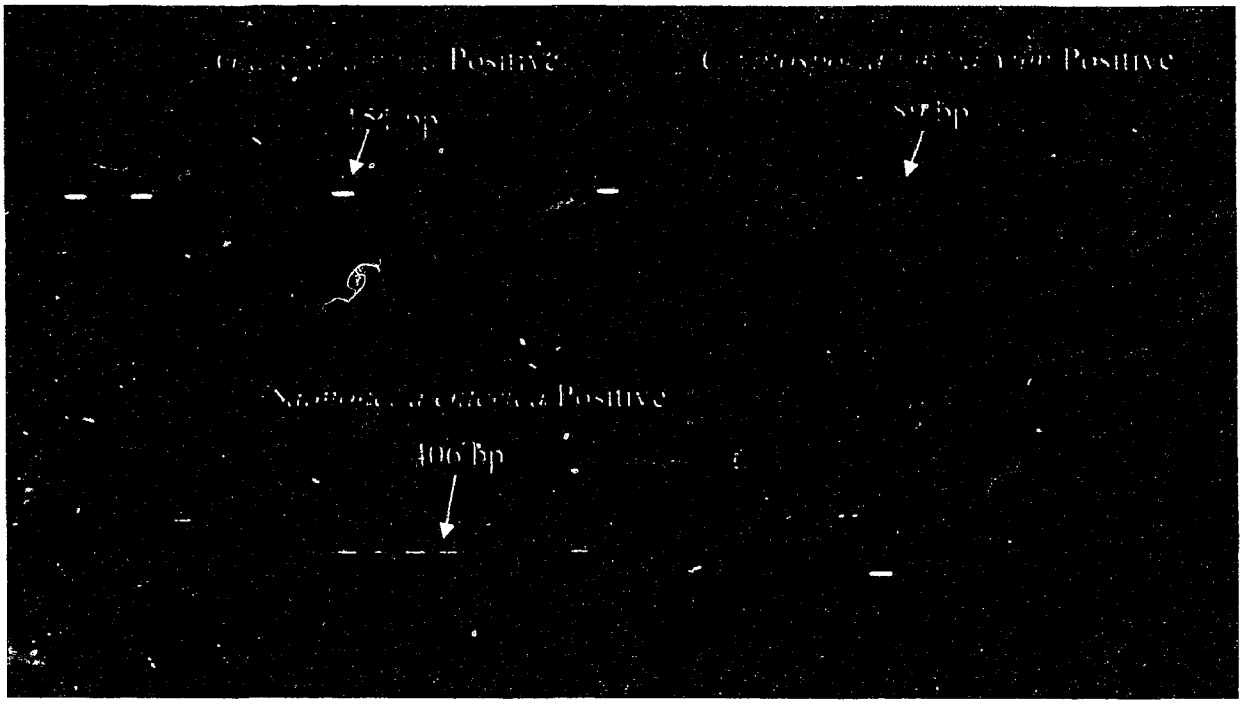


**Figure 3-6**: Agarose gel depicting *Giardia lamblia, Cryptosporidium parvum,* and *Salmonella enterica* positive samples.

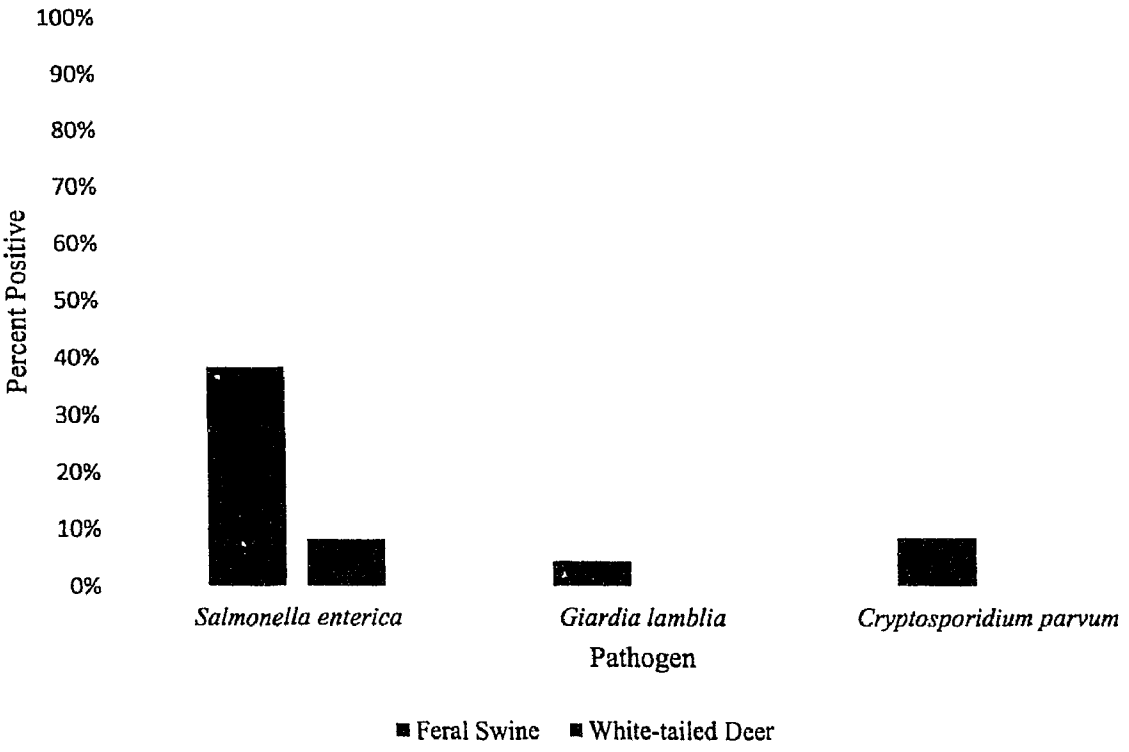


**Figure 3-7**: Percentage of feral swine and white-tailed deer fecal samples that tested positive for *Salmonella enterica, Giardia lamblia*, and *Cryptosporidium parvum*.

Each fecal sample that tested positive for *Cryptosporidium parvum* DNA also showed intact oocysts when analyzed using microscopy. Figure 3-8 shows an example of a *Cryptosporidium parvum* oocyst.

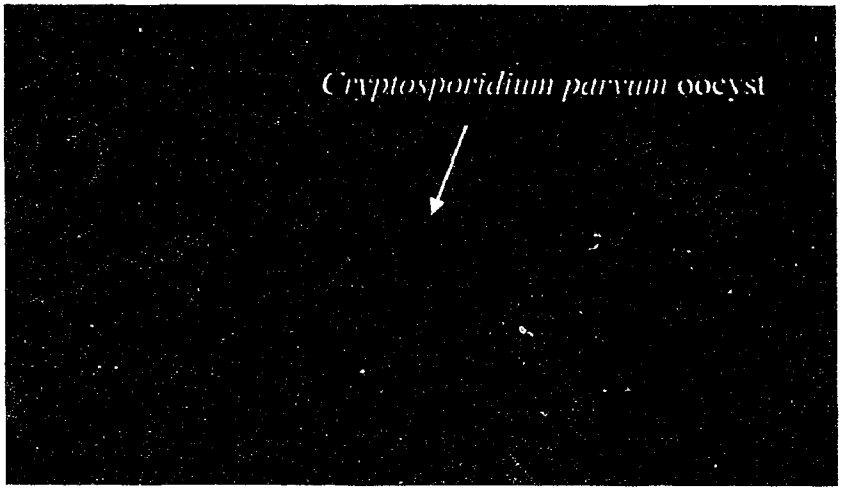


**Figure 3-8:** Microscopic image of a *Cryptosporidium parvum* oocyst.

### 3.5 Feral Swine Wallow Analysis

The dissolved oxygen levels in the wallows we sampled ranged from less than 1 ppm to over 4 ppm. The pH of our wallow samples ranged below 4 pH to above 6 pH. The temperature ranges of the wallows we sampled was 79°F to 89°F (Figures 3-9 through 3-11). XRF analysis showed that the levels of biologically relevant heavy elements in feral swine wallow sediment were similar to those found in sediment not disturbed by feral swine. XRF analysis also showed there was sufficient levels of iron to support microbial growth (Figure 3-12). These results show that the abiotic conditions of the wallows are suitable for all pathogens that were tested for in this study.

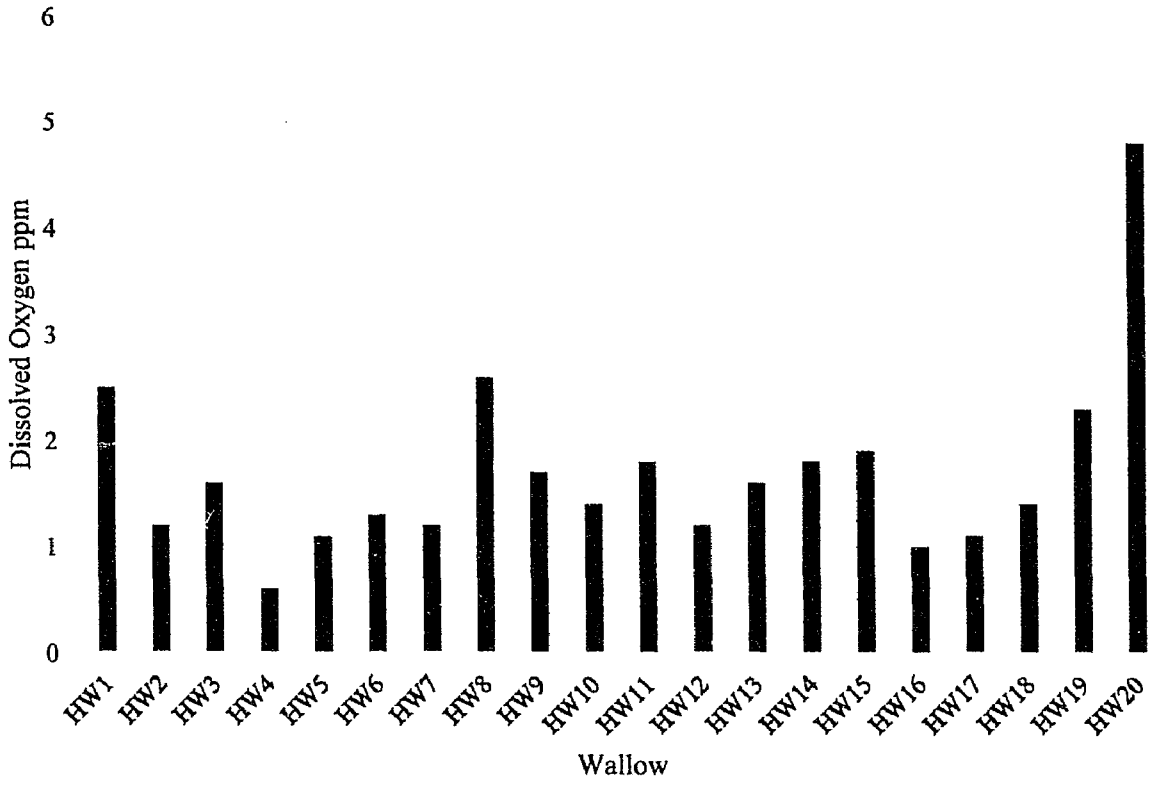


**Figure 3-9**: Dissolved oxygen levels in feral swine wallows (n=20), "HW" in sample names seen along the X-axis designates "Hog Wallow".

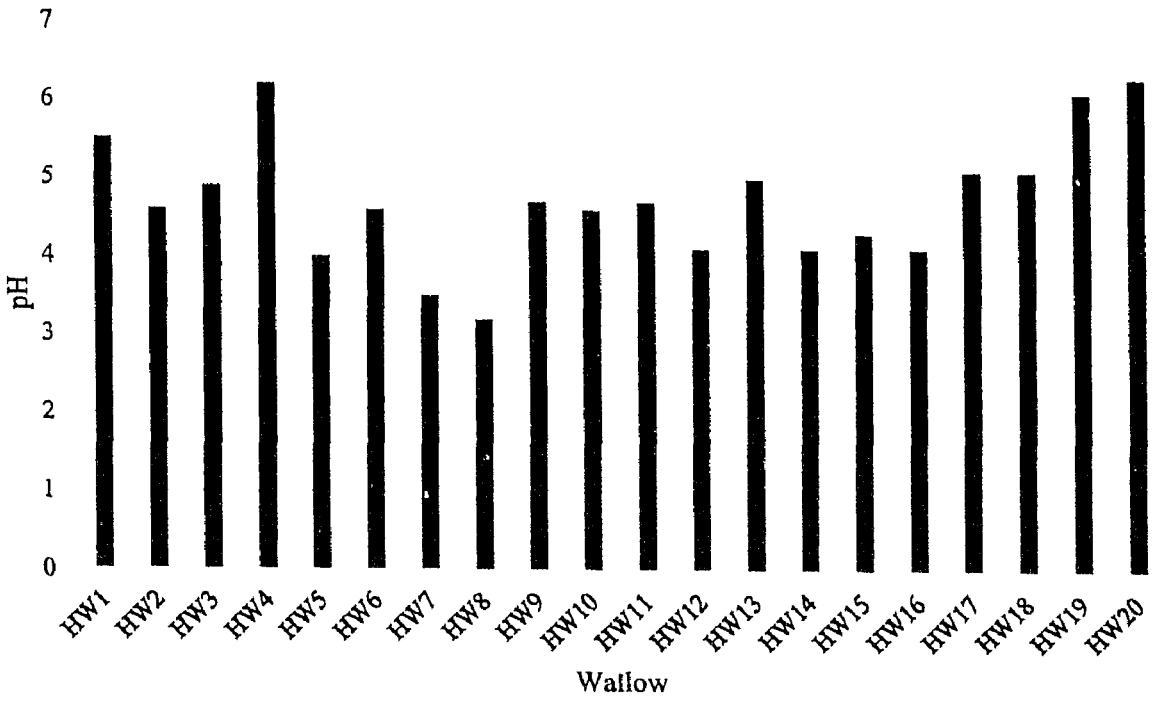


**Figure 3-10**: pH levels in feral swine wallow samples (n=20).

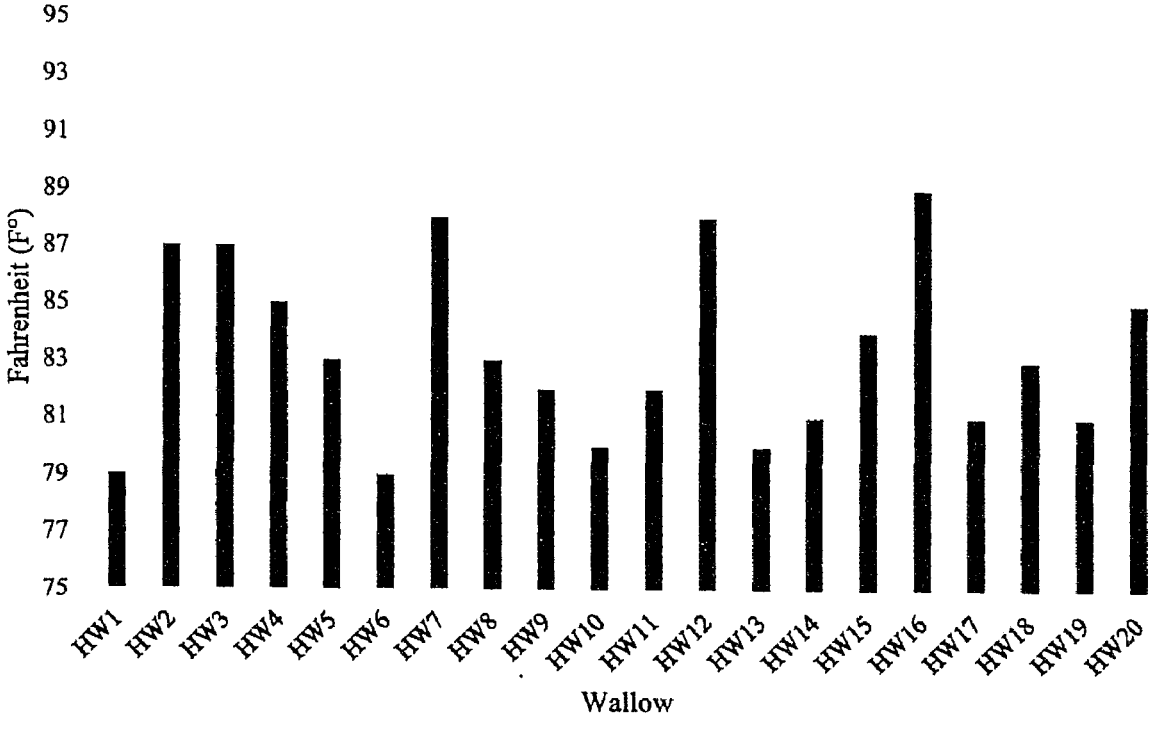


**Figure 3-11**: Temperatures of feral swine wallow samples (n=20).

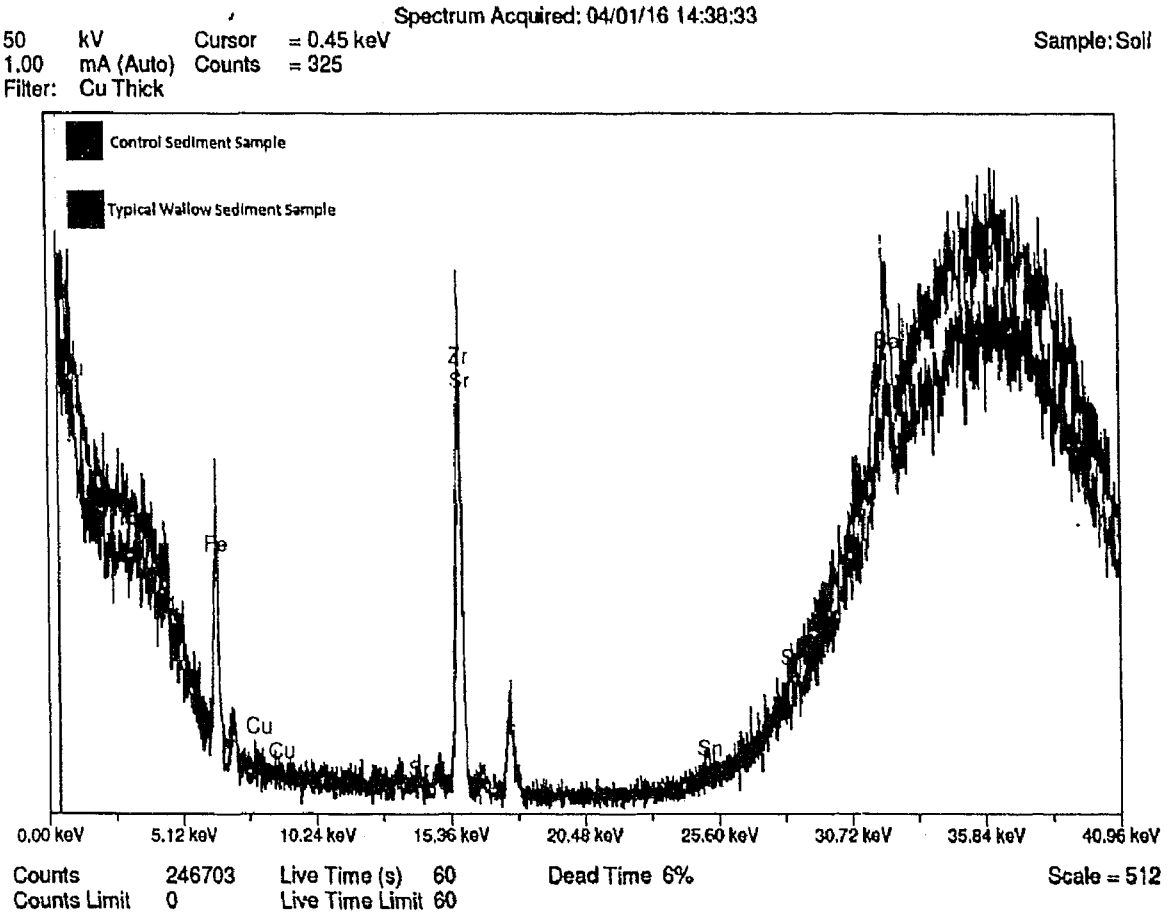


**Figure 3-12**: XRF spectrum showing a comparison of levels of heavy elements in a control sediment sample and a sediment sample from a feral swine wallow.

All wallow samples showed high levels of fecal coliform, indicating a high level of fecal contamination. None of the wallow samples tested positive for either human or ruminant specific *Bacteroides*. All samples tested positive for pig specific *Bacteroides*, indicating the source of bacterial contamination was feral swine. The results of our PCR analysis detected *Brucella* spp. DNA in 30% (6 of 20) of the wallows tested. Seventy-five percent (15 of 20) of the wallows tested positive for *Leptospira interrogans* DNA. Sixty-five percent (13 of 20) wallows tested positive for *Salmonella enterica. Cryptosporidium*

*parvum* and *Giardia lamblia* were detected in 20% (4 of 20) and 25% (5 of 20) of wallow samples respectively. Table 2 and Figure 3-13 show a summary of this data.

**Table 2**: Summary of Feral Swine Wallow Coliform Counts and Presence of Pathogens

| Wallow | Coliforms/100 ml | *Brucella* Positive | *L. interrogans* Positive | *S. enterica* Positive | *C. parvum* Positive | *G. lamblia* Positive |
|---|---|---|---|---|---|---|
| HW1 | 2,100 | No | Yes | Yes | Yes | No |
| HW2 | 14,000 | Yes | Yes | Yes | No | Yes |
| HW3 | 27,000 | No | No | Yes | Yes | No |
| HW4 | 1,300 | No | Yes | No | No | No |
| HW5 | 500 | No | Yes | Yes | No | No |
| HW6 | 7,000 | Yes | Yes | Yes | No | Yes |
| HW7 | 3,000 | No | Yes | Yes | No | No |
| HW8 | 23,000 | No | Yes | Yes | No | No |
| HW9 | 59,000 | No | No | Yes | Yes | Yes |
| HW10 | 3,400 | No | Yes | Yes | No | No |
| HW11 | 58,000 | Yes | Yes | Yes | No | Yes |
| HW12 | 4,100 | Yes | Yes | No | No | No |
| HW13 | 14,000 | No | No | Yes | No | No |
| HW14 | 60,000 | No | Yes | Yes | No | Yes |
| HW15 | 2,300 | Yes | Yes | No | No | No |
| HW16 | 2,600 | No | Yes | No | No | No |
| HW17 | 1,200 | No | Yes | Yes | Yes | No |
| HW18 | 700 | No | No | No | No | No |

**Table 2**: Summary of Feral Swine Wallow Coliform Counts and Presence of Pathogens (continued).

| Wallow | Coliforms/100 ml | *Brucella* Positive | *L. interrogans* Positive | *S. enterica* Positive | *C. parvum* Positive | *G. lamblia* Positive |
|---|---|---|---|---|---|---|
| HW19 | 98 | No | Yes | No | No | No |
| HW20 | 85 | Yes | No | No | No | No |

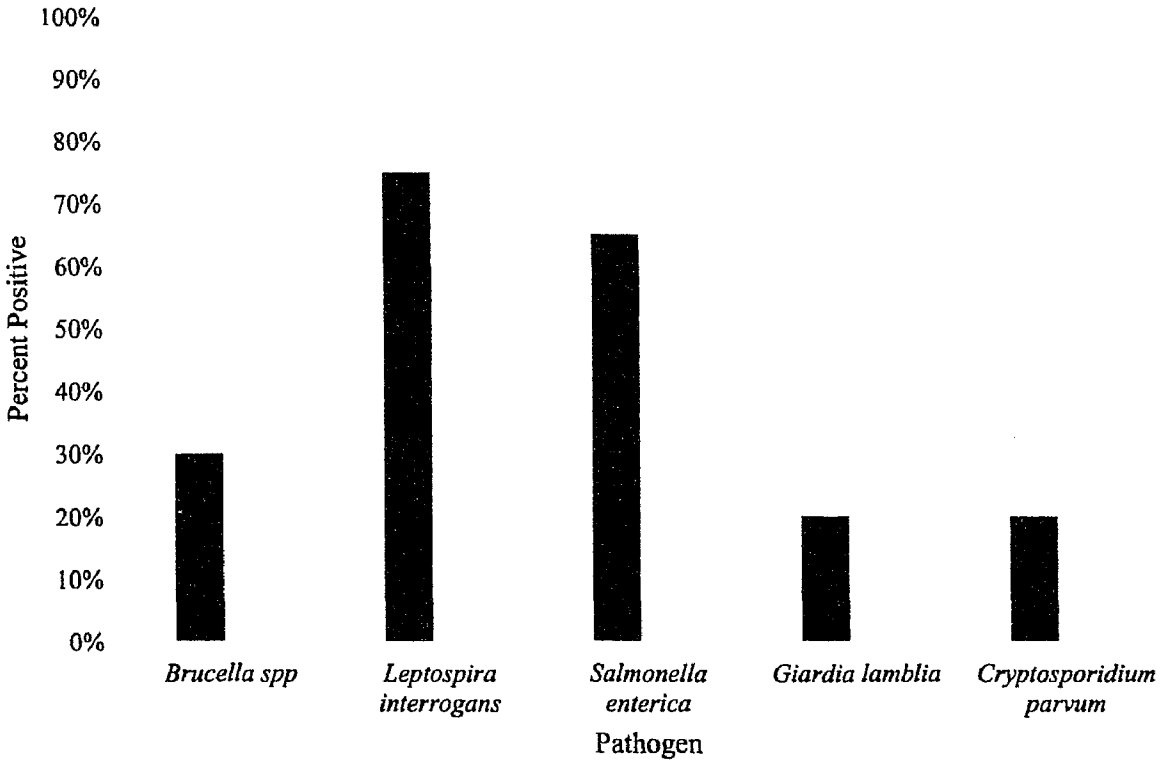


**Figure 3-13**: Percentage of feral swine wallows that tested positive for each pathogen.

### 3.5.1 Locations of Captured Feral Swine and Feral Swine Wallows

Figures 3-14 through 3-18 show the locations of captured feral swine and feral swine wallows that were included in this study. Each map also shows where each feral swine and wallow that tested positive for a specific pathogen was located. Feral swine

wallows are depicted by the label HW followed by a corresponding number that identifies which feral swine wallow it is. The locations of our white-tailed deer samples were unable to be identified due to the nature of how those samples were collected. These maps were generated using the ARCGIS program.

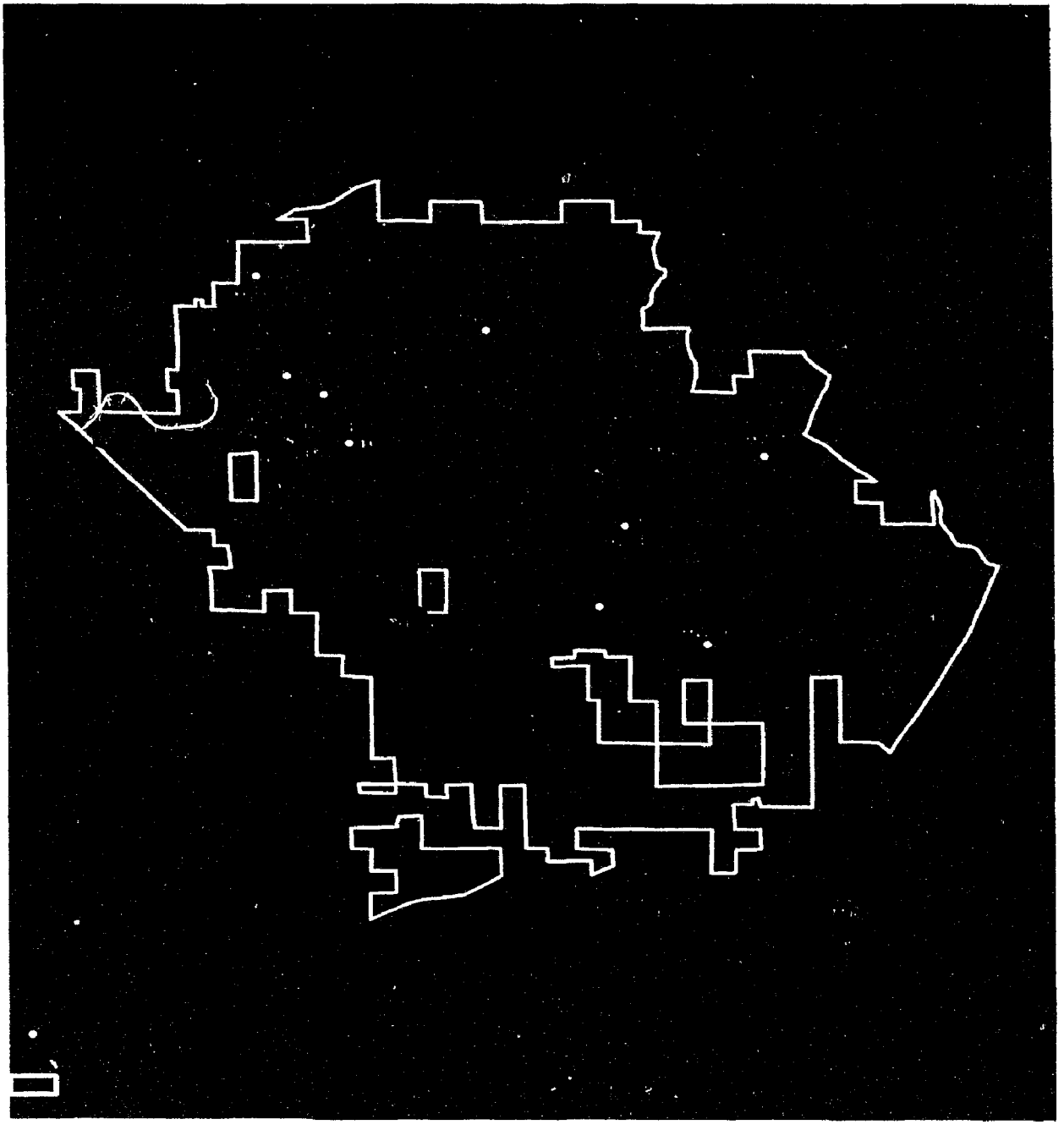

**Figure 3-14**: Locations of feral swine and feral swine wallows that tested positive for *Brucella* spp.

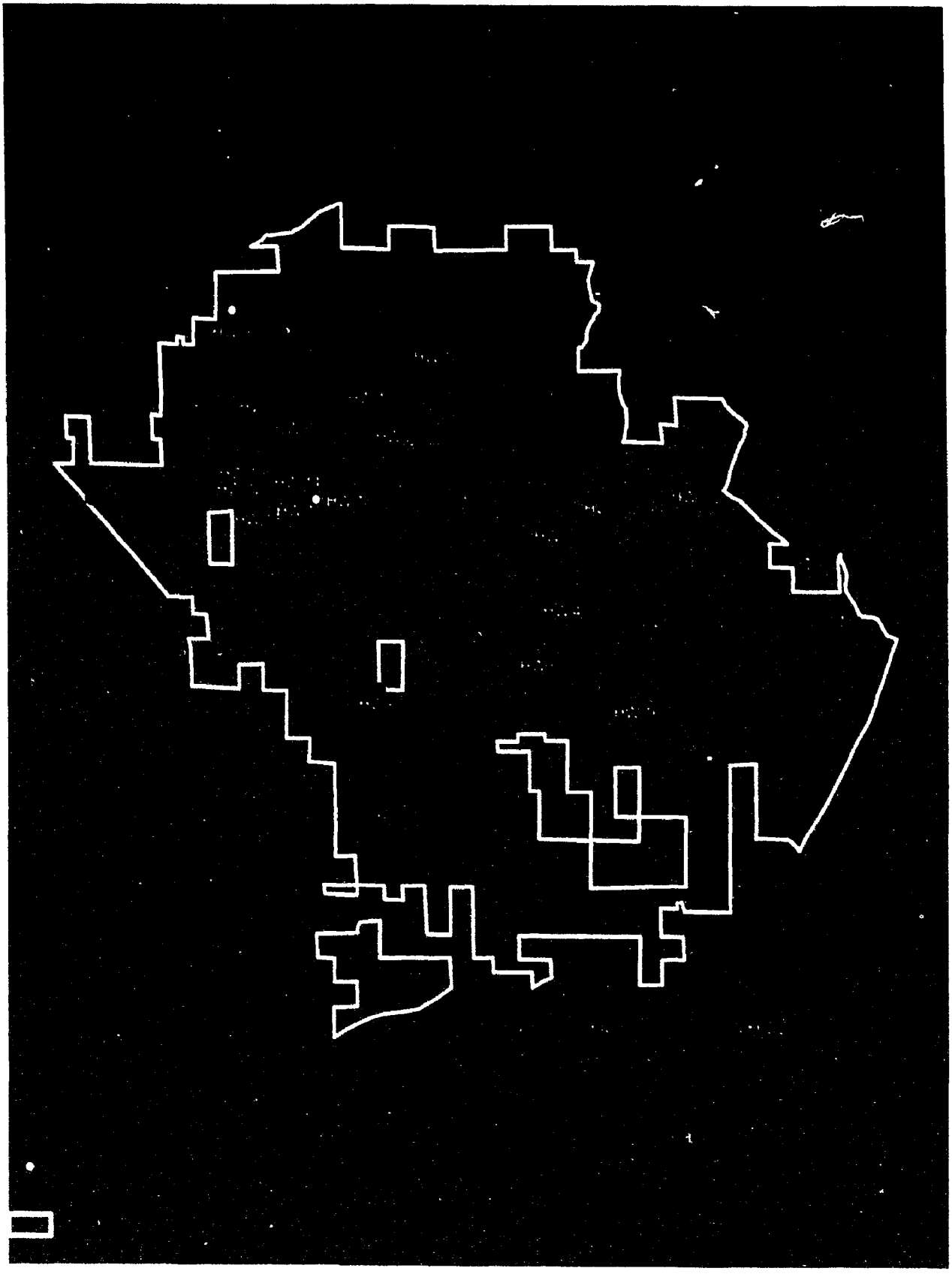

**Figure 3-15**: Locations of feral swine and feral swine wallows that tested positive for *Leptospira interrogans*.

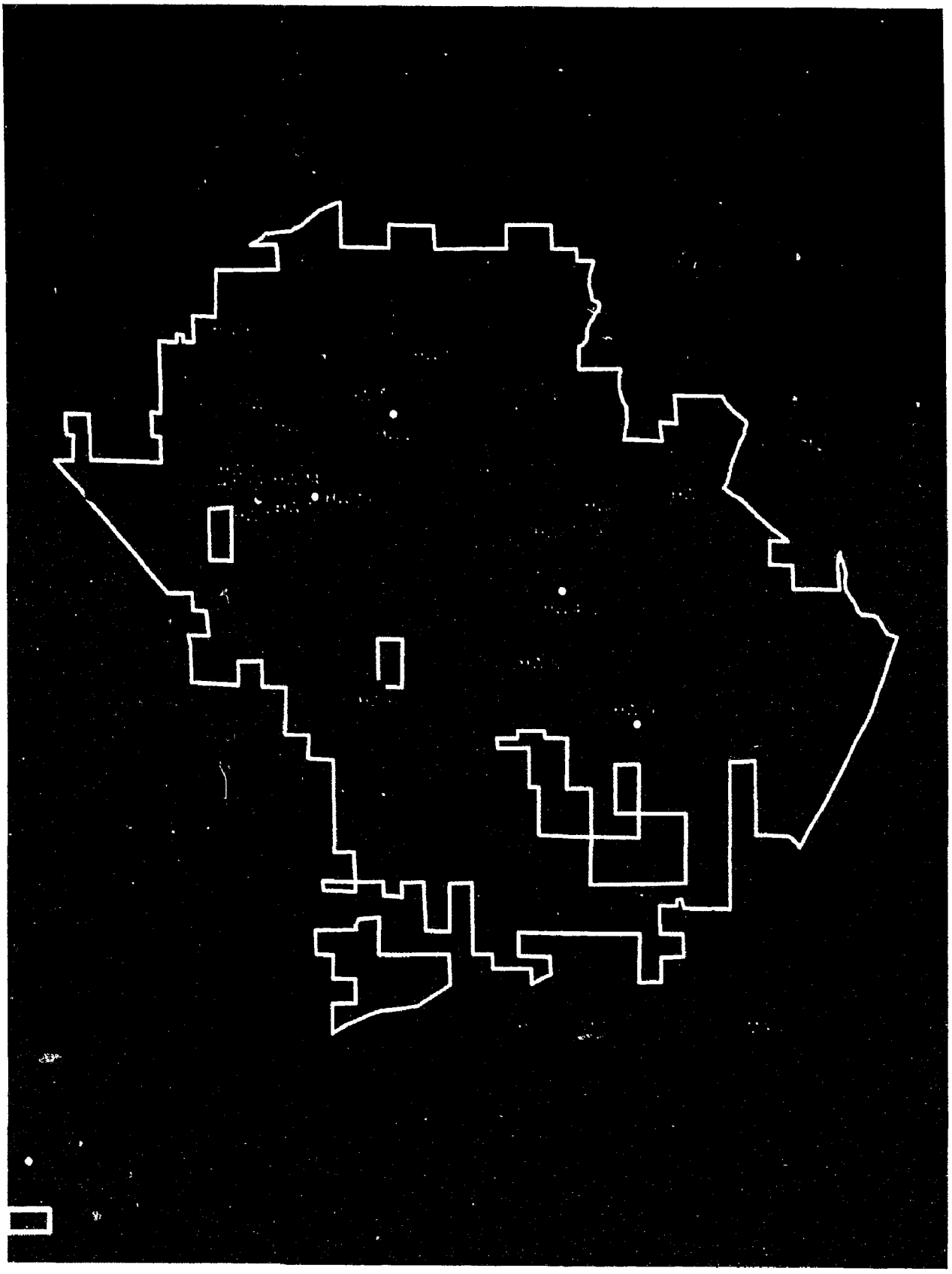

**Figure 3-16**: Locations of feral swine and feral swine wallows that tested positive for *Salmonella enterica*.

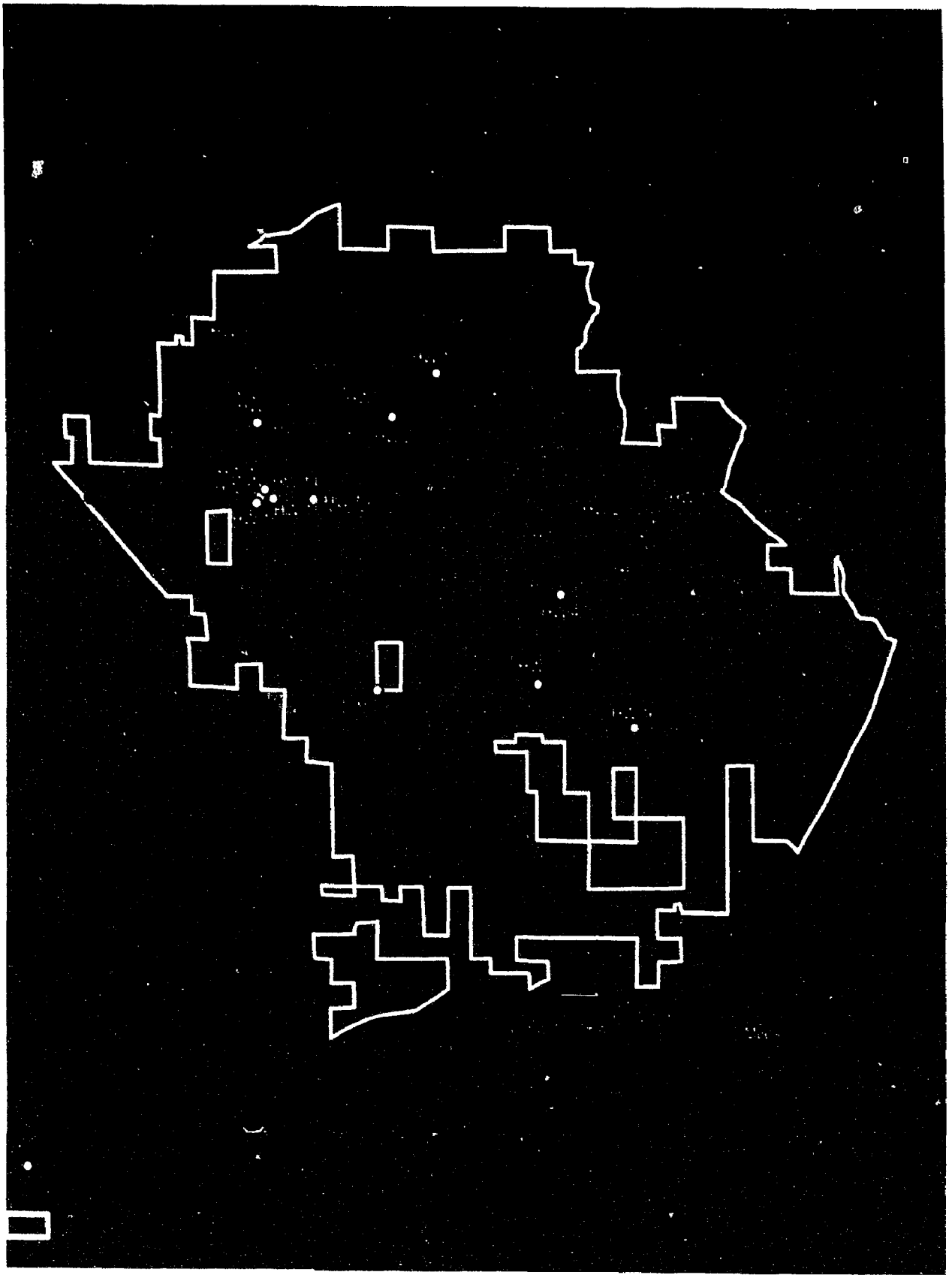

**Figure 3-17**: Locations of feral swine and feral swine wallows that tested positive for *Cryptosporidium parvum*.

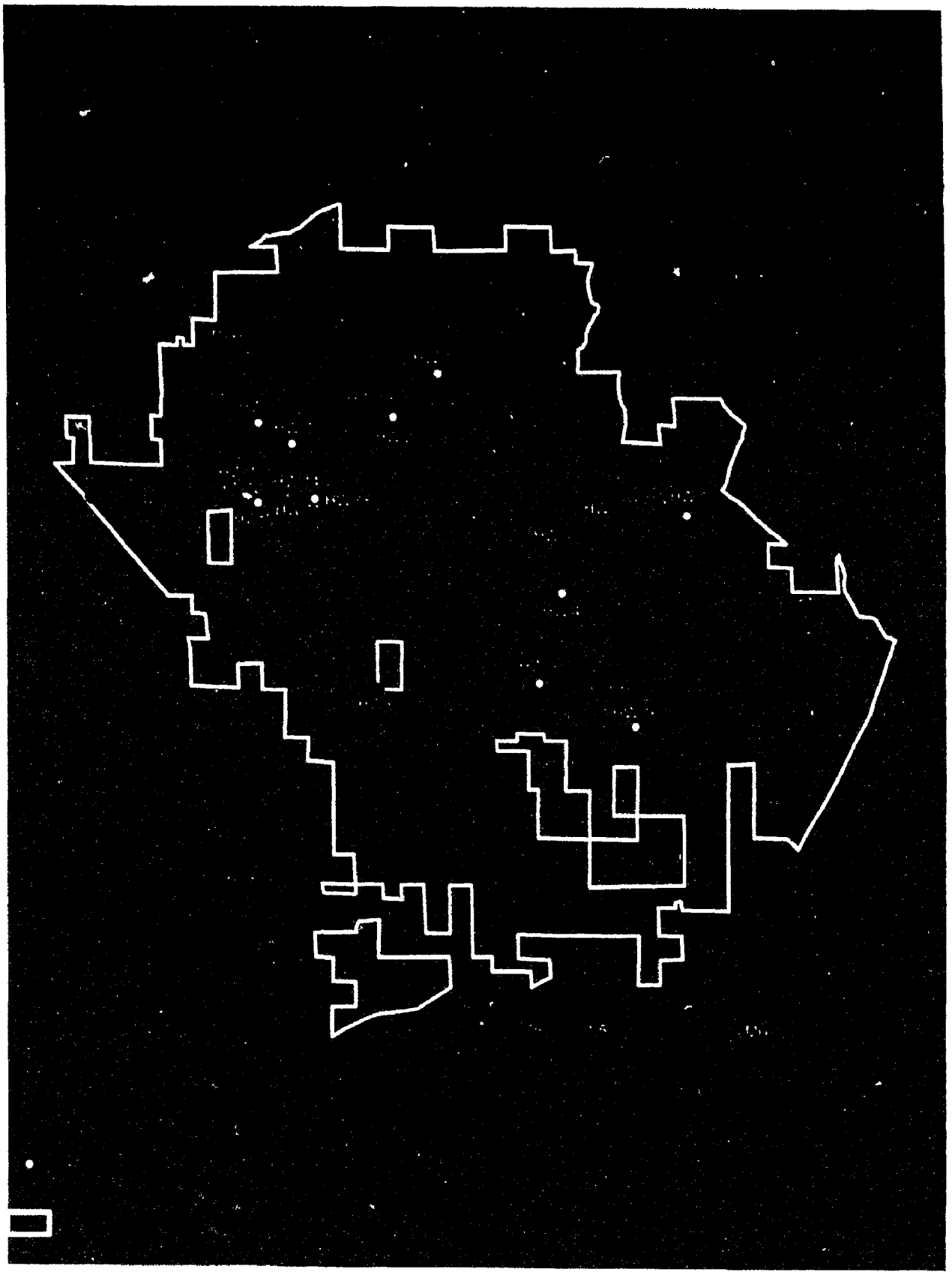

**Figure 3-18**: Locations of feral swine and feral swine wallows that tested positive for *Giardia lamblia*.

### 3.6 Characteristics of Collected Feral Swine and Whitetail Deer Samples

We recorded the age, weight, and gender of all feral swine and white-tailed deer samples in order to calculate if there were any correlations between these variables and pathogen prevalence. The age of feral swine ranged from 6 months to 4 years. The age range of whitetail deer was from 6 months to 7 years (Figures 3-19 and 3-20). The weight range of feral swine was anywhere from 4 pounds. to 238 pounds. Whitetail deer ranged from 55 lbs. to 190 lbs. (Figures 3-21 and 3-22). The ratio of males to females in both the feral swine and whitetail deer that were collected was nearly 1:1 (Figures 3-23 and 3-24).

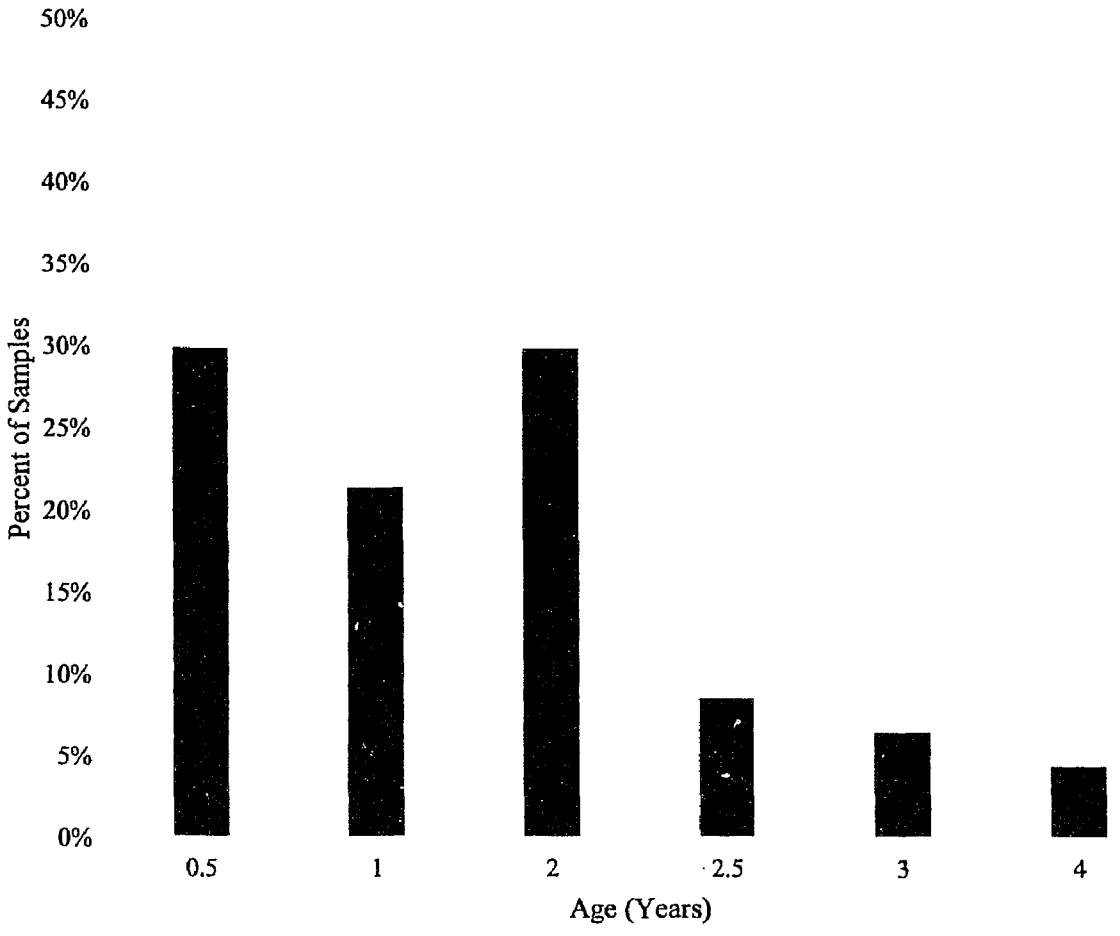


**Figure 3-19**: Age structure of feral swine included in this study (n=47).

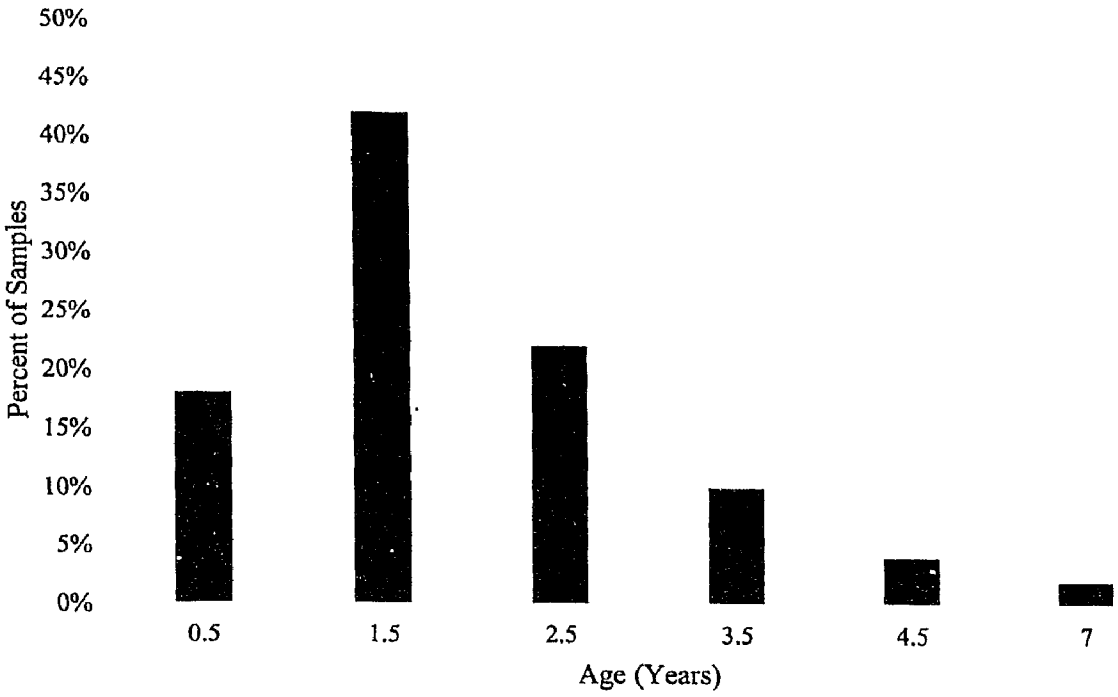


**Figure 3-20**: Age structure of white-tailed deer included in this study (n=49).

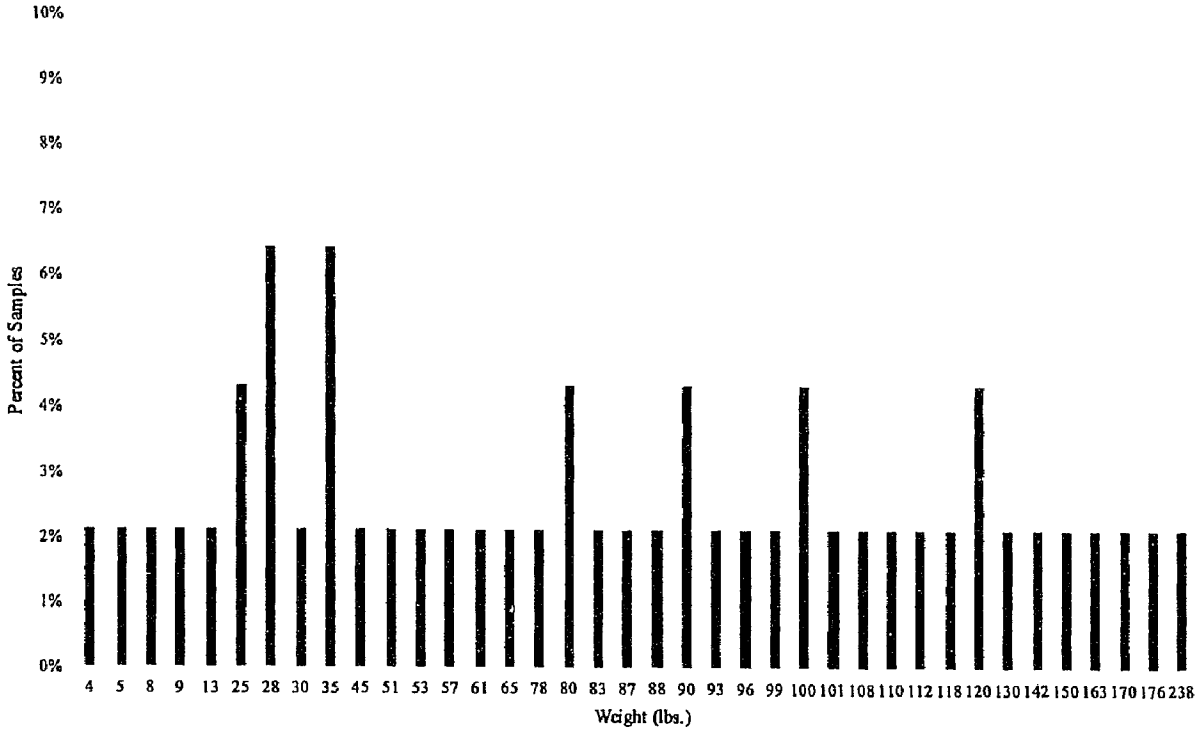


**Figure 3-21**: Weight distribution of feral swine included in this study (n=47).

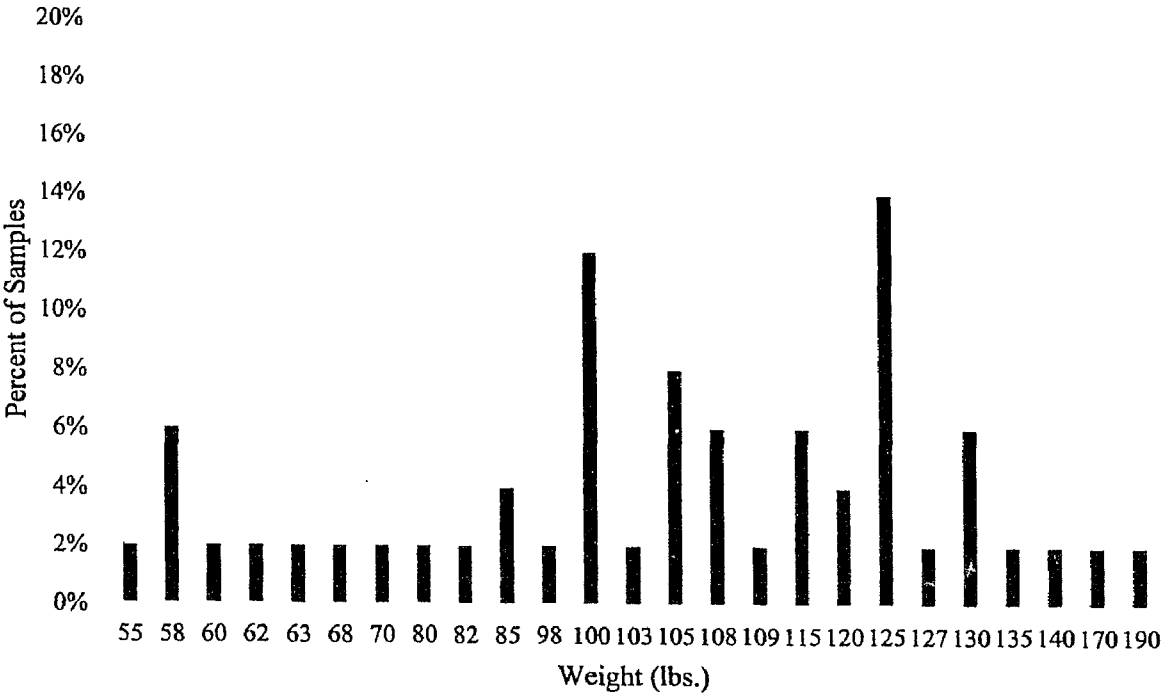


**Figure 3-22**: Weight distribution of white-tailed deer included in this study (n=49).

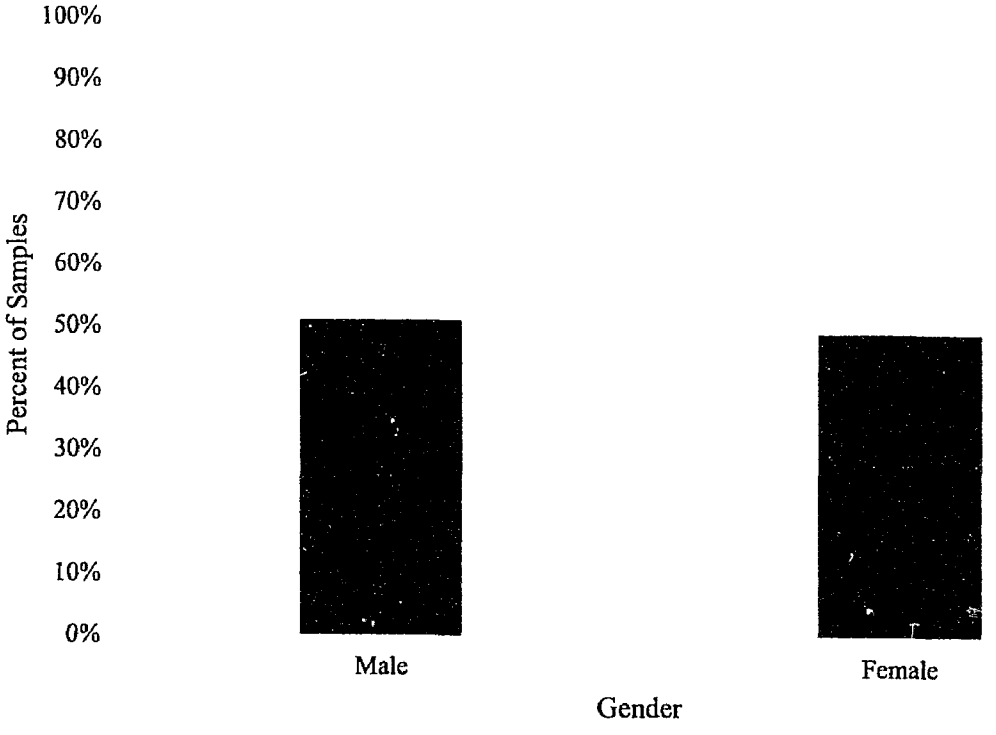


**Figure 3-23**: Percentage of male and female feral swine included in this study (n=47).

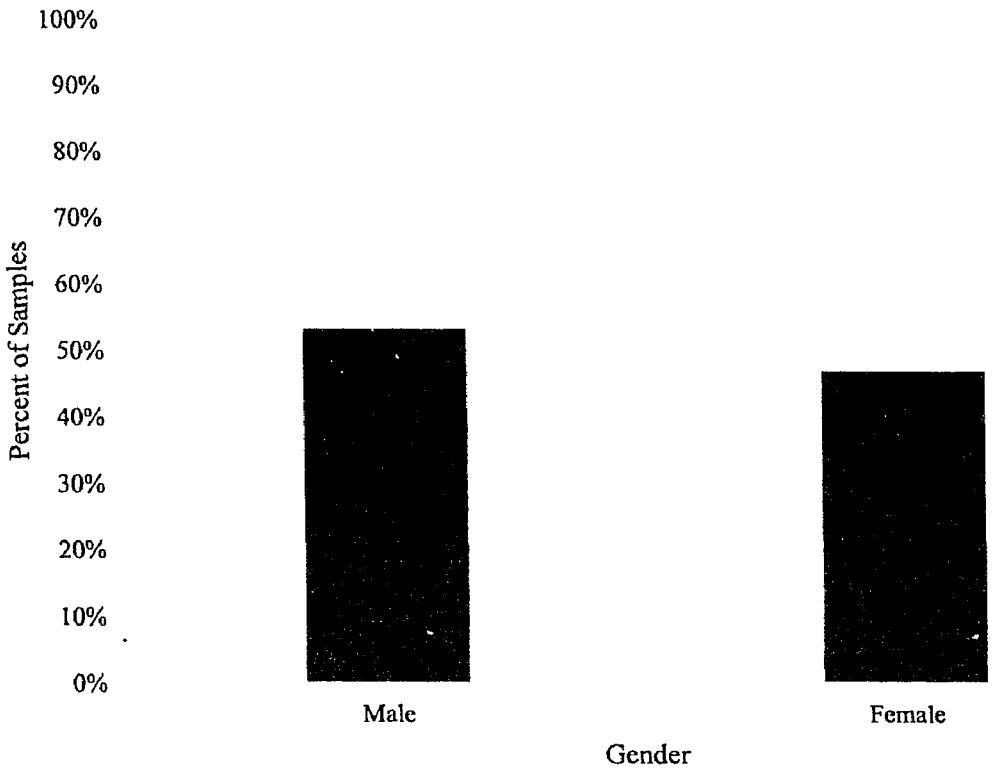


**Figure 3-24**: Percentage of male and female white-tailed deer included in this study.

## 3.7 Correlations between Age, Weight, or Gender and Pathogen Prevalence

The results from the binomial logistic regression test that was performed showed that there was only one statistically significant relationship between the age, weight, or gender of feral swine and white-tailed deer and pathogen prevalence (Table 3). There was a statistically significant relationship between feral swine weight and *Leptospira interrogans* exposure (p-value 0.039). The less a feral swine weighed the greater the chance for *Leptospira interrogans* exposure.

**Table 3**: Binomial Logistic Regression for Comparison of Age, Weight, and Gender and *Leptospira interrogans* Exposure

| Variables in the Equation | | | | |
|---|---|---|---|---|
| | | Sig. (p-value) | 95% C.I. for EXP(B) | |
| | | | Lower | Upper |
| Step 1[a] | Age | .516 | .075 | 3.675 |
| | Gender(1) | .782 | .211 | 3.229 |
| | Weight | .039 | .998 | 1.085 |
| a. Variable(s) entered on step 1: Age, Gender, Weight. | | | | |

### 3.8 Correlations between Coliform Count and Pathogen Prevalence in Wallows

The results from the binomial logistic regression that was performed on the data for coliform counts and pathogen prevalence in feral swine wallows showed that there was no statistically significant correlation between coliform counts and pathogen prevalence.

# CHAPTER 4

# FERAL SWINE POPULATION ESTIMATION IN THE JACKSON BIENVILLE WILDLIFE MANAGEMENT AREA

## 4.1 Introduction

Infectious disease has been shown to have a major influence on population dynamics and the distribution of many species (77). Many species of wildlife have become more susceptible to disease over the years due to habitat destruction, introduction of invasive species, and human displacement of animals (78). Some of these displaced or invasive species are reservoirs for diseases that can pose a serious threat to the survival of native wildlife. This can have profound consequences in areas that rely on native wildlife for economic reasons, such as national parks and other tourist destinations. There are also risks to human health when diseased wildlife is prevalent in the environment. These reasons present financial and clinical motivations for studying how diseases affect wildlife populations. Understanding the dynamics of how disease impacts wildlife is critical to preventing and maintaining healthy wildlife populations.

One of the consequences of disease in wildlife populations that has only recently been investigated is how disease impacts the physical characteristics of wildlife populations, including gender ratios, weight distribution, and age structure (78). These traits play a critical role in the survivability and viability of a population. If a disease disproportionately affects females it can negatively affect overall population growth.

Diseases that mainly affect the young can also have a negative effect on population growth and the overall viability of a population. With this in mind our group conducted a population study in the J-B WMA to determine if any correlations exist between age, weight, and/or gender in feral swine and whitetail deer and the prevalence of particular pathogens. This knowledge can aid in controlling the spread of these pathogens as well as equipping local wildlife biologists with information that can be used to manage the population of feral swine and whitetail deer in the J-B WMA.

## 4.2 Methods

Research conducted by David Stafford estimated that approximately 550 feral swine were present on the J-B WMA during the time of this study. Camera traps were used to capture images of feral swine during the summer of 2015. The Jacobson method was used to analyze the data obtained from his camera study (79), which is a modified form of the mark-recapture technique for estimating population size that compensates for the use of cameras. A portion of the population is captured and marked, or in this case photographed, and released. Another sample group is photographed and the number of unique individuals from the first group is counted. Since the number of unique individuals within the second group is assumed to be proportional to the number of unique individuals in the entire population, one can estimate the total population size. This is done by dividing the number of unique individuals by the proportion of unique individuals in the second sample. This survey uses cameras to capture, distinctly spotted hogs as marked individuals, and to recapture marked individuals. Figure 4-1 shows the location of the camera traps used in his study.

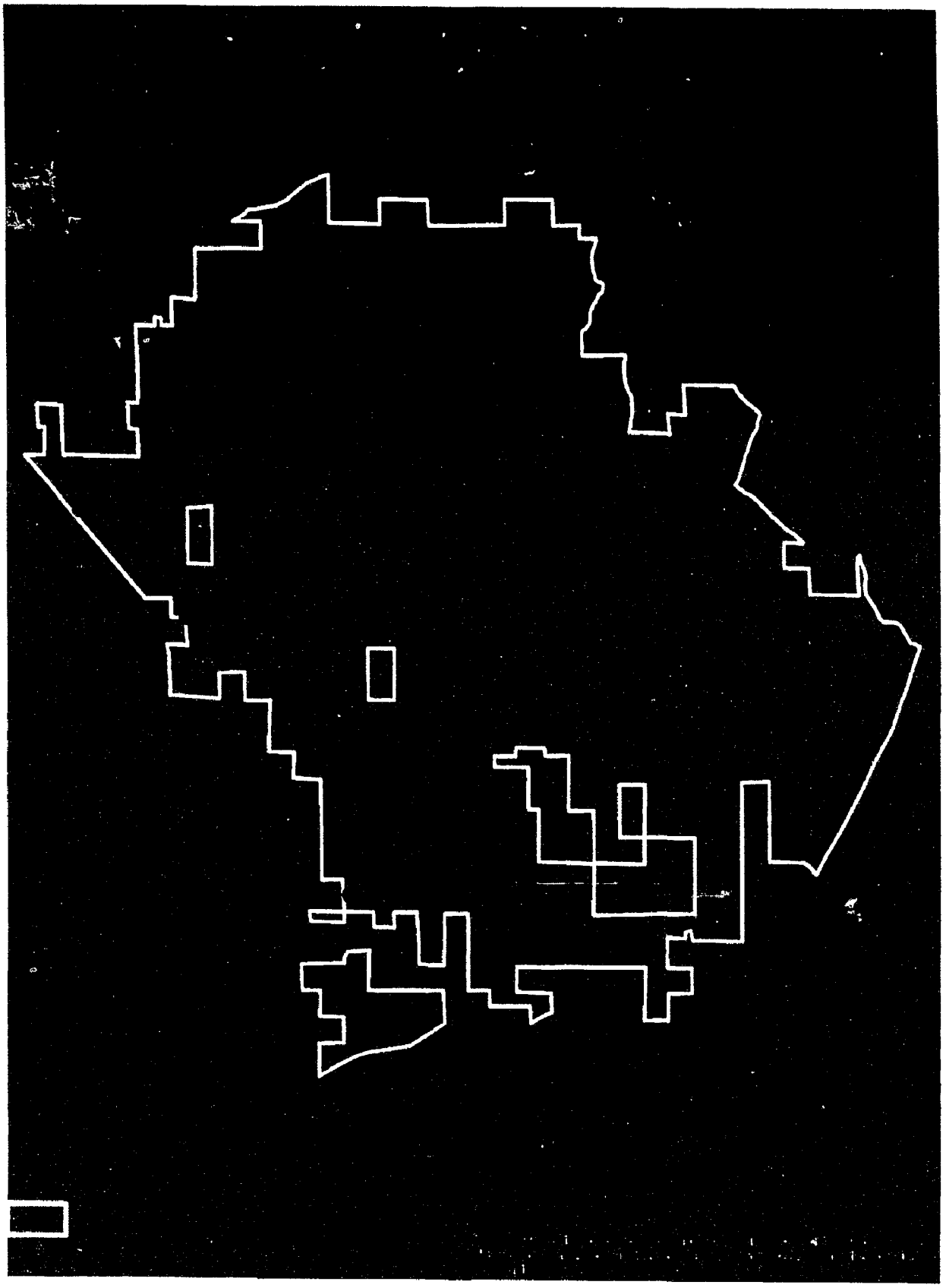

**Figure 4-1**: Location of camera traps used in the population study conducted by David Stafford.

## 4.3 Results

The population size of feral swine in the J-B WMA was estimated to be between 500-600 individuals based on data obtained from David's camera study. Based on these results, we were able to determine that our sample size of feral swine used for this part of the study was approximately 10% of the overall population of feral swine in the J-B WMA.

# CHAPTER 5

# DISCUSSION AND FUTURE WORK

## 1.1 Discussion

These results provide evidence indicating that a significant number of feral swine are carrying pathogens with potential to cause disease in both humans and wildlife. The feral swine in the J-B WMA are depositing these pathogens into shallow wallows, and in the process potentially contaminating the watershed and directly exposing any humans or wildlife to these pathogens. The temperature, pH, dissolved oxygen, and elemental composition of the wallows showed that they contain the necessary environment to sustain bacterial and protozoal growth. However, it is not known how long pathogens can persist in the wallows, and our study did not analyze the overall watershed. Regardless, the fact we were able to detect the presence of DNA from a number of protozoal and bacterial pathogens means there is a potential for these pathogens to either spread directly to humans and wildlife, or contaminate streams and larger bodies of water with which they communicate.

Testing for the presence of *Brucella* spp. in feral swine yielded higher positive percentages compared to past records (verbal communication from James LaCour, D. V.M., Louisiana State Wildlife Veterinarian, unpublished data) indicating a possible increase in the overall spread of this pathogen in this feral swine population. Although

previous research has shown a correlation between population density and *Brucella* spp. prevalence, there was no discernable pattern seen when the locations of infected feral swine were plotted out. More data is needed over a longer period of time for the J-B WMA to determine if such a correlation exists.

The seroprevalence of *Leptospira interrogans* observed in this study was alarmingly high compared to historical data, but is consistent with current state levels (James LaCour, D.V.M., unpublished data). The most alarming aspect was the number of feral swine that tested positive for exposure to the hardjo serovar. The hardjo serovar was also responsible for 5 of the 6 active infections detected. Current vaccines for leptospirosis do not protect against the hardjo serovar, which could lead to an increased risk for infection even in animals that have been vaccinated. Although the MAT assay is the gold standard for detecting exposure to *Leptospira interrogans*, we corroborated those results by using PCR. The PCR results agreed with over 85% of what was found in the MAT assay. This is most likely due to the increased sensitivity of PCR. There appeared to be a cluster pattern of *Leptospira interrogans* infected feral swine after plotting their locations. *Leptospira interrogans* contaminated wallows also showed a similar pattern. This lends evidence to how easily *Leptospira interrogans* can spread in groups of feral swine.

*Salmonella enterica* was found in a large number of feral swine tested, as well as a large number of wallows. This is concerning due to how easily *Salmonella enterica* can spread and how long it can persist in the environment. Such a high prevalence of this pathogen poses a significant risk for human infection and watershed contamination. Although *Salmonella enterica* does not cause chronic or life threatening diseases, it still

poses a significant risk to human health. The location of PCR positive feral swine and wallows appeared to be evenly spread throughout the J-B WMA.

*Cryptosporidium parvum* and *Giardia lamblia* were both found in a low number of feral swine and wallows. This is not surprising if we assume feral swine are currently a minor reservoir for both of these pathogens on the J-B WMA. *Giardia lamblia* is primarily found in beavers, with feral swine being a secondary reservoir. *Cryptosporidium parvum* is mostly found in cattle and other ruminants. Despite this, these results demonstrate that deer are not currently posing a problem as a reservoir in the J-B WMA.

Overall, whitetail deer showed a low prevalence for most pathogens in this study. The exception to this was *Brucella* spp. The percentage of *Brucella* spp. positive whitetail deer was nearly identical to the percentage of positive feral swine, with similar sample sizes (47 feral swine, 49 whitetail deer). This study also showed that a high number of feral swine wallows tested positive for *Brucella* spp., and indicated a possibility for feral swine to potentially spread this pathogen through wallow water. This leads one to suspect that feral swine could potentially be the source of *Brucella* spp. exposure we saw in whitetail deer.

Another interesting result was the seroprevalence of only one serovar of *Leptospira interrogans* in our whitetail deer samples, serovar hardjo. This serovar also had the highest prevalence in feral swine. These results are alarming due to the fact that none of the vaccines for leptospirosis protect against this serovar. These results are also interesting due to the hardjo serovar being relatively uncommon in this part of the United

States. These results support the argument that whitetail deer were exposed to *Leptospira interrogans* by feral swine.

*Salmonella enterica* was found in very few whitetail deer. These results are not unexpected and are in line with historical data on the prevalence of *Salmonella enterica* in whitetail deer. There is very little evidence to suggest that feral swine are the source of *Salmonella enterica* infection in this population of whitetail deer. *Cryptosporidium parvum* and *Giardia lamblia* were not found in any of the whitetail deer samples tested. This is interesting due to whitetail deer being a significant reservoir for *Cryptosporidium parvum*. This, combined with low numbers of these pathogens in feral swine, could mean that these two pathogens are not very prevalent in the J-B WMA.

The distribution of gender and weight were fairly even for both feral swine and whitetail deer. The age distribution was skewed toward the lower end of the age range for both feral swine and whitetail deer. The low number of positive samples for all pathogens except *Leptospira interrogans* is most likely why there was only one correlation found between the variables tested and pathogen prevalence.

The results of the camera study David Stafford conducted showed that our sample size for feral swine was roughly 10% of the overall population. This meant our data met the basic assumptions suitable for using parametric statistics, which allowed us to use binomial logistic regressions to analyze our data.

## 5.2 Conclusions

This study represents a unique ecological snapshot of the current status of six waterborne pathogens within a defined study area as they occur in two large mammal species and an aquatic reservoir. Our results can serve as a foundation for future studies

to examine dynamic interactions of these pathogens with these hosts and the environment, particularly the watershed. An interesting hypothesis to test would be the potential flow of pathogens from a high incidence in the feral hog population and onto other species via wallows and contamination of the watershed.

## 5.3 Future Work

Recommendations for future work in this area should include collecting a larger sample size over a longer period of time to better represent trends of how these pathogens are being spread. Many of the short comings of this study involved accurately detecting the presence of *Brucella* spp. Future studies should include the use of ELISA assays to increase the accuracy of detecting exposure to this pathogen. Genotyping of pathogens found in feral swine and whitetail deer would be able to provide strong evidence for determining a probable source of infections.

We also recommend that this study be expanded to include serological surveys of hunters that hunt on the J-B WMA. This would determine if direct exposure to feral swine and/or whitetail deer is facilitating the spread of these pathogens. Initially, a serological survey should be done to quantify hunter exposure to *Leptospira interrogans*. This particular pathogen was by far the most prevalent found in this ecosystem. If positives are identified, a follow up could include PCR detection of pathogens as well as pathogen genotyping to determine their source. While genotyping may not definitively show a causal relationship between the feral swine reservoir and downstream species, it would provide a strong foundation to conduct future studies on the exact nature of the interrelationships occurring between hosts and pathogens in the study area.

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